For Reference

NOT TO BE TAKEN FROM THIS ROOM

UNIVERSITY OF ALBERTA
LIBRARY

Regulations Regarding Theses and Dissertations

Typescript copies of theses and dissertations for Master's and Doctor's degrees deposited in the University of Alberta Library, as the official Copy of the Faculty of Graduate Studies, may be consulted in the Reference Reading Room only.

A second copy is on deposit in the Department under whose supervision the work was done. Some Departments are willing to loan their copy to libraries, through the inter-library loan service of the University of Alberta Library.

These theses and dissertations are to be used only with due regard to the rights of the author. Written permission of the author and of the Department must be obtained through the University of Alberta Library when extended passages are copied. When permission has been granted, acknowledgement must appear in the published work.

This thesis or dissertation has been used in accordance with the above regulations by the persons listed below. The borrowing library is obligated to secure the signature of each user.

Please sign below:
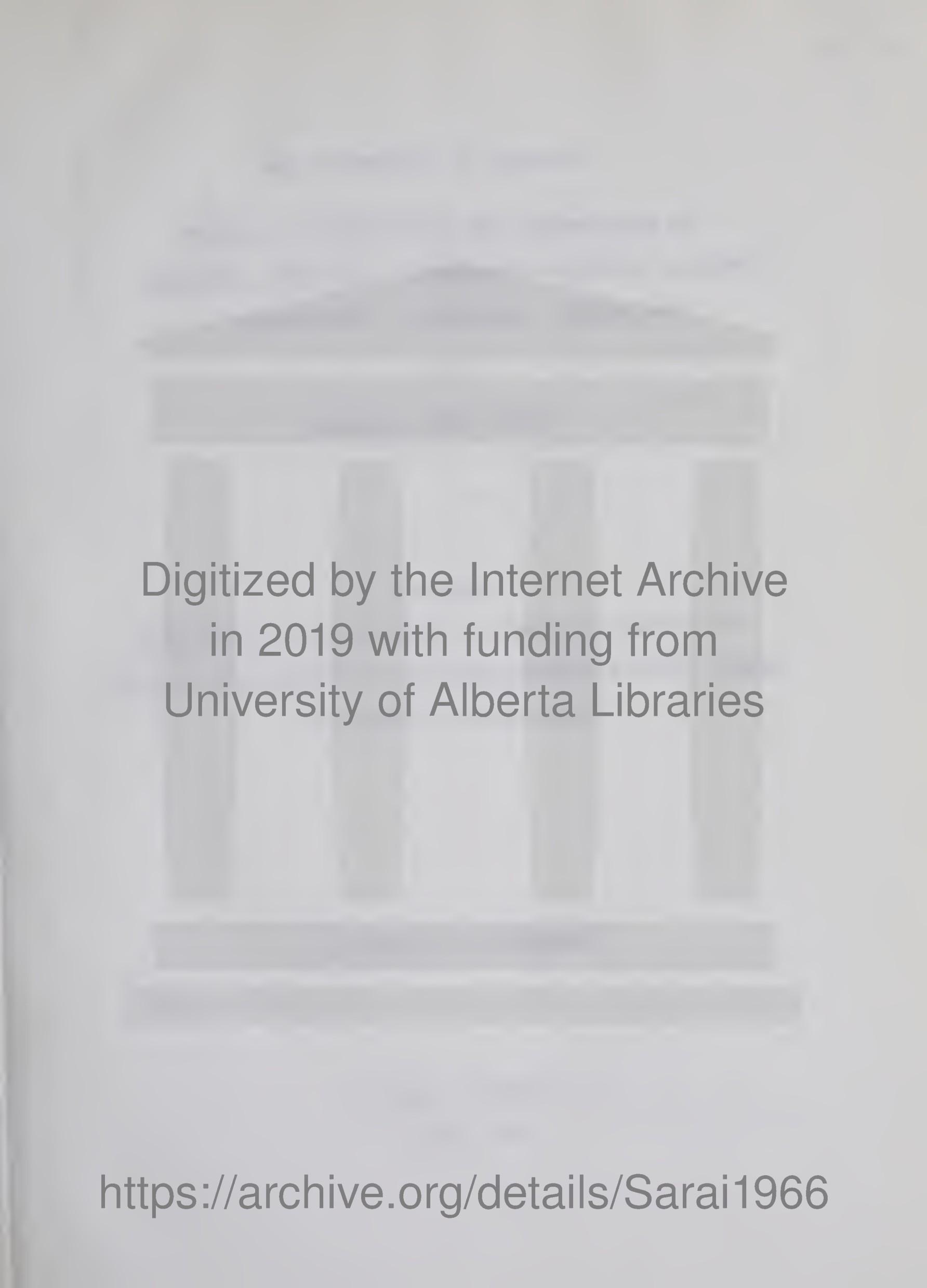
Date   Signature   Institution

I

THE UNIVERSITY OF ALBERTA

EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON

EMBRYONIC DIAPAUSE IN NEMOBIUS FASCIATUS (DEGEER)

(ORTHOPTERA: GRYLLIDAE: NEMOBIINAE)

by

DARSHAN SINGH SARAI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

EDMONTON, ALBERTA

APRIL, 1966

Date: April 25, 1966

UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Effects of Photoperiod and Temperature on Embryonic diapause in Nemobius fasciatus (DeGeer) (Orthoptera: Gryllidae: Nemobiinae) submitted by Darshan Singh Sarai in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Date: April 25, 1966

ABSTRACT

The effects of temperature and photoperiod on invoking and terminating embryonic diapause of <u>Nemobius</u> <u>fasciatus</u> were studied in relation to the adaptation of this cricket to the long winter of a continental climate at 53° - 30'N.

<u>N.</u> <u>fasciatus</u> is univoltine in the region of Alberta. Eggs which undergo facultative diapause at the termination of anatrepsis overwinter and hatch in early July of the succeeding year. There are six nymphal instars; these mature early in August and the adults survive until frosts occur in late October. Diapause is induced chiefly by wide fluctuations in day and night temperatures in late summer. Middle aged females deposit higher percentages of diapausing eggs than younger or older females. A high incubation temperature (29°C) suppresses diapause. Diapause is terminated by exposing pre-diapausing or diapausing eggs to low temperatures (0 - 15°C) before incubation. Heavy mortality occurs, however, in freshly laid eggs when they are exposed to 0°C. Diapause is terminated after 96 days exposure to 5°C for different incubation temperatures (20 - 29°C). A low temperature of 5°C is not harmful to post-diapause eggs except for cessation of development which is resumed within 24 hours at an incubation temperature of 29°C. Exposure of any stage to constant or varying photoperiod does not influence incidence of diapause.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. <u>INTRODUCTION</u>

Adaptations such as a univoltine life cycle (single generation per year) and resistence to low temperature, at least in the resting stages, are commonly found in insects which inhabit high northern latitudes. In these regions climatic variations which are responsible for these adaptations are very wide. In the resting stage, developmental processes and metabolism in general are very much retarded. This phenomenon of physiological rest, which is known as diapause, is followed by an active period of the life cycle at the onset of favourable conditions. Because these favourable conditions exist only for a short period of the year in the temperate regions, most of the insects existing there are univoltine (Danilyevsky, 1965). On the other hand, in regions where favourable conditions remain for longer periods, insects are mostly multivoltine (more than one generation per year). Diapause in univoltine insects is mostly obligatory and in multivoltine species facultative (Andrewartha and Birch, 1954; Lees, 1955; and Danilyevsky, 1965). In the former, diapause is commonly invoked by internal physiological processes regardless of the external conditions, but the life cycle as a whole remains under the influence of temperature which determines the breaking of diapause and also the period of development of the active stages. In multivoltine insects facultative diapause is mainly controlled by external factors, such as

photoperiod, temperature, food and humidity (Lees, 1955; and
Danilyevsky, 1965).

It was once believed by some authors (Readio, 1931;
Cousin, 1932) that diapause was invoked by unfavourable con-
ditions, in most insects.   But the principal stimulus for
the inception of diapause is photoperiod, although temper-
ature, water and diet may be involved (Harvey,1962).   Al-
though photoperiod has no direct effect on the development
of insects, it serves to influence the neurosecretory system
which enables them to enter diapause at the proper time of
the year so as to survive unfavourable conditions.   The
discovery of the dependence of the inception of diapause on
photoperiod was mainly the work of Kogure (1933) on certain
bivoltine races of _Bombyx_ _mori_ L. in Japan.   Recent works
include those of Danilyevsky (1948, 1949 and 1951, cited in
Danilyevsky,1965), Dickson (1949), and Lees (1955).

The present study was undertaken to determine how
Nemobius _fasciatus_, the striped ground cricket, is adapted to
inhabit a part of North America which is one of the coldest
locations in the known distribution of this species.   As
this adaptation is mainly through embryonic diapause, this
study is concerned with the effect of environmental factors
on induction and termination of diapause.   This species is
omnivorous like most crickets, feeding on decaying organic
matter, and leaves of grasses.   Diet was discarded as a
possible environmental factor effecting diapause because

crickets are general feeders.   Similarly the possibility of
water was eliminated, because eggs remain in moist soil from
the time of oviposition to hatching.   Temperature and photo-
period were, therefore, studied in different combinations to
determine their effects on the induction and termination of
diapause in this insect.

2. NOTES ON SYNONYMY

Nemobius fasciatus (DeGeer)

Gryllus fasciatus DeGeer 1773: p.552.

Acheta hospes Fabricius 1775: p.281.

Acheta vittata Harris 1831: p.123.

Nemobius vittatus, Scudder 1868: p.3.

Nemobius fasciatus socius Scudder 1877: p.37. Fulton 1931: p.223. Cantrall 1943: p.163. Pierce 1948: p.129.

Nemobius fasciatus vittatus, Allard 1910: p.354.

DeGeer described this cricket in 1773 from Pennsylvania. Thereafter different authors described it under different names from various parts of North America, particularly from the eastern United States. There was a great deal of confusion among three closely related species of *Nemobius* which were taken by some authors as sub-species of *Nemobius fasciatus* until Alexander and Thomas (1959) reviewed the literature and examined pinned specimens from various localities determined previously by different workers. They differentiated these species into *Nemobius fasciatus* (DeGeer), *Nemobius allardi* Alexander and Thomas and *Nemobius tinnulus* Fulton basing their classification particularly on song variation and also on some morphological characters such as length of ovipositor, number of teeth on the stridulatory

vein, shape and size of head capsule.

Before this work was undertaken, this species was not recorded in Alberta, perhaps it had been found but mis-identified. Hebard (1930) mentioned _Nemobius fasciatus fasciatus_ (DeGeer) (which is now _Nemobius allardi_ Alexander and Thomas) in Alberta and described its limit as probably meeting that of _Nemobius fasciatus abortivus_ Caudell (now _Nemobius abortivus_ Caudell) in south eastern Alberta or in north eastern Montana. Alexander and Thomas (1959) stated that Hebard confused both _Nemobius fasciatus_ (DeGeer) and _Nemobius allardi_ Alexander and Thomas in his discussion of these species. Thus it is possible that _Nemobius fasciatus fasciatus_ (DeGeer) of Hebard in Alberta is _Nemobius fasciatus_. The crickets from the vicinity of Edmonton were very kindly determined by Dr. R.D. Alexander to whom eight male and female specimens with notes on habitat and a tape recording of the song were sent for this purpose.

3. GENERAL METHODS

Crickets used in various experiments were collected in 1964 in hummocky pastures near Atim Creek. In 1965, however, another meadow about two and a half miles away from Atim Creek was used as the collection site, because of the flooding of the previously used field. Crickets break their metathoracic legs easily if netted and in order to collect them uninjured, adults and last instar nymphs were caught individually with a wide-mouthed fruit jar. The mouth of the jar was put in front of the cricket and the latter was then induced to jump into the jar. By this method I sometimes collected 80 crickets in one hour. As males sing while sitting in the grass, it was easy to locate them by their sound. Each male was commonly found with one or two females which were probably attracted by the song. Young nymphs were collected in the field with a trapping aspirator. Crickets thus brought from the field were kept in the laboratory in battery jars with rabbit pellets for food (Ghouri and McFarlane, 1958), inverted bottles of water plugged with absorbent cotton for water supply and strips of paper towels to increase the surface area for walking and for hiding. About 20 to 30 crickets in equal sex ratio were kept in each jar. Fresh moist soil in plastic petri dishes was provided for oviposition each 24 hours. Eggs were sieved out underwater

in a tray and damaged or undersized ones were discarded. They were then put in plastic petri dishes on a filter paper covering a thick pad of wet absorbent cotton. These petri dishes were always kept covered by plastic covers to avoid evaporation of water, except for a few minutes every day for observation. Covering of the petri dishes is essential, because, eggs are highly susceptible to desiccation. The cotton remained moist for about three weeks at 29° and 24°C in the covered petri dishes. At lower temperatures such as 0, 5 and 15°C the water lasted for two months. Whenever it was necessary, a few drops of tap water were put on the filter paper. Each petri dish was marked both on the cover and on the undersurface to indicate the number of the experiment, the number of eggs and date of oviposition, etc.

Eggs were observed after 24 hours for hatching. Newly hatched nymphs were removed from the dish with an aspirator and counted by putting them in a fruit jar. To check this counting, egg shells were also counted in the dish and removed from among the rest of the eggs with a pair of forceps.

Eggs for laboratory experiments were deposited by the crickets which were collected in the field mainly in the first half of September, since ovipositing adults were found in abundance at that time of the season. Eggs laid within 24 hours of the collection of the crickets were used.

The experiments of 1964 were repeated, under similar conditions, in 1965 on comparable dates (unless otherwise stated).

Hogan's method (1959) for _Acheta_ _commodus_ Walker, a cricket found in Australia, was used to determine the stage of embryonic development at which diapause occurs in the eggs of _N._ _fasciatus_. The eggs were soaked in water in a watch glass for half an hour. The water was then drained off and replaced by a mixture of two parts of glacial acetic acid, two parts of chloroform and one part of absolute alcohol for 25 minutes at 34°C. Eggs were then transferred to a mixture of one part of glycerol and one part of 70 percent alcohol. This method proved satisfactory for optical differentiation of the embryo, which was clearly observed under the microscope without cutting sections or dissecting eggs.

The percentage hatching of eggs shown in the results are those which hatched within 13 to 15 days at 29 ± 1°C and within 20 to 22 days at 24°C, for non-diapausing or post-diapausing eggs. This criterion was applied because it was seen throughout this study that these were the incubation periods for development without diapause at these incubation temperatures.

4. FIELD OBSERVATIONS AND DEVELOPMENTAL HISTORY

4.1 Introduction

N. fasciatus is recorded in wet localities over almost all of eastern North America including Newfoundland and Florida.   By putting together various records of its distribution (Lugger, 1897; Blatchley, 1920; Fulton, 1931; Hebard, 1930, 1936; Strohecker, 1937; Ball et al, 1942; Alexander and Thomas, 1959; Vickery, 1963) it is found that this species is distributed from Southern Canada to Northern Mexico and east of the great basin in the United States. Characteristically, it inhabits marsh borders and other poorly drained grassy situations such as stream banks, meadows and pastures.   It is commonly associated with crab grass (Digitaria sanguinalis Scop.).

In the northern United States this cricket over-winters in the egg stage, matures in July and continues to sing until the last individuals are killed by the oncoming winter.   In Ohio, the earliest recorded singing date is July 8 and the latest one is November 14.   In the University of Michigan Museum there are adult specimens from central and northern localities of Michigan, the earliest of which was collected on July 17, the latest on November 16.   There are also adult specimens which were taken in Florida every month of the year (Alexander and Thomas, 1959).   N. fasciatus starts

maturing before the middle of June in the Piedmont area and coastal plains of North Carolina and probably there are two to three generations per year at this latitude (Fulton, 1931).

Near Edmonton, however, for two summers (1964 and 1965) the songs of this cricket were not heard before the first week of August or after the last week of October.

4.2 Seasonal history in Edmonton

Observations were made on the seasonal history of N. fasciatus in meadows near Atim Creek, about 21 miles west of Edmonton, from the end of April to the end of October, both in 1964 and 1965.   In 1964 the summer was normal and there was hatching of nymphs from July 8 to the 15.   Mature specimens were observed for the first time on July 30, but there was no singing even though the day was warm (22.7°C) and singing starts a day or so after the last molt.   A week later, the buzzy chirps were very noticeable in the field.   Oviposition had started by the 10th of August, and reached a maximum between the last week in August and the middle of September (Fig. 1).   Oviposition was poor when it remained cool for most of the time (Sept 5 to 11).   On August 27, 5th and 6th instar nymphs were still present but they were seen for the last time on October 10.   Heavy frosts in the third week of October killed most of the crickets
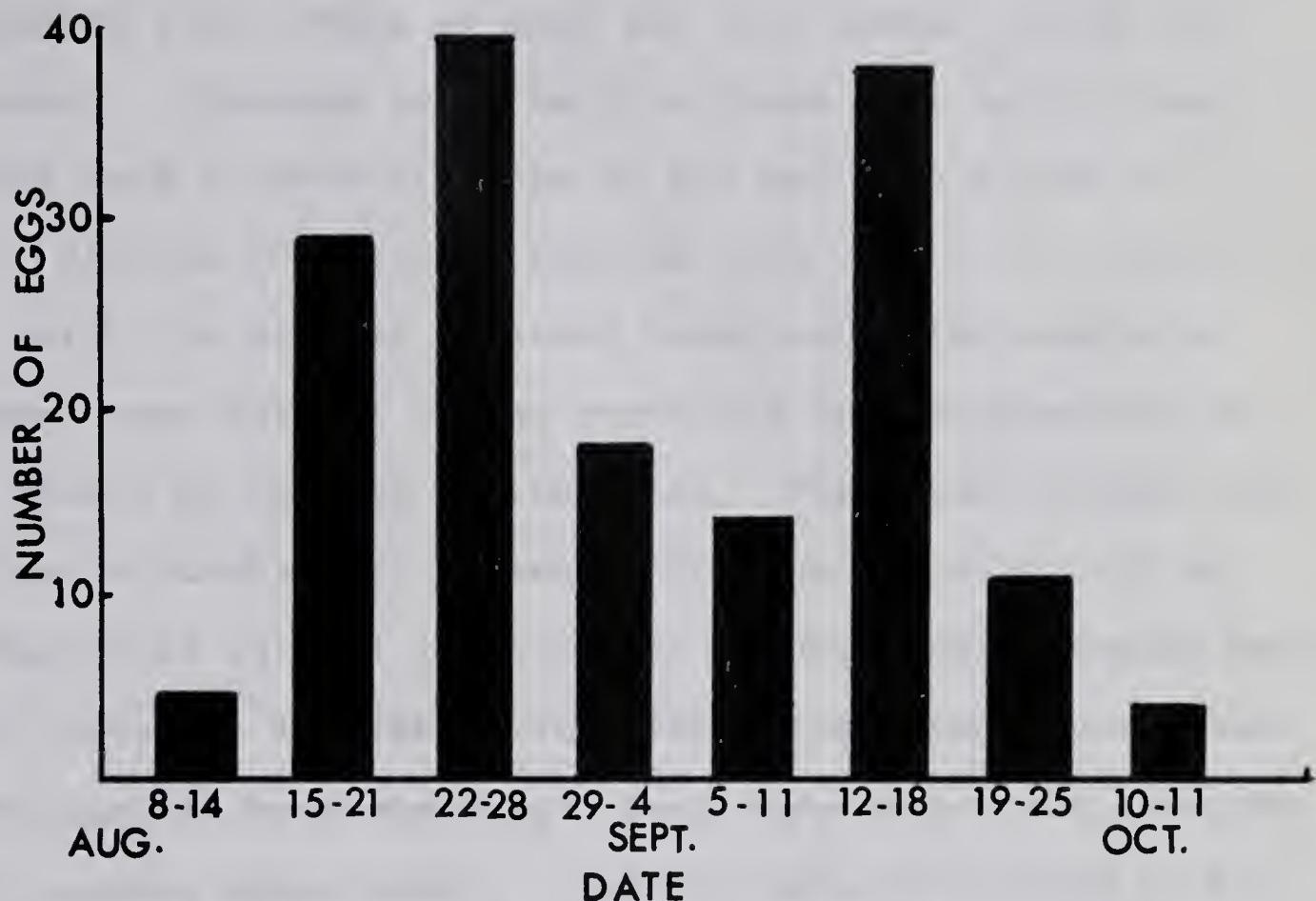
Figure 1. Number of eggs deposited by

N. fasciatus per week per female caged outside

during the summer 1964.

and they were heard last on October 24, which was a partly
sunny day with temperature 11.6°C in the afternoon.

In 1965 there were 7.48 inches of rainfall in June
compared to 1.04 inches in 1964 and 3.15 inches normal for
this month.   Meadows near the Atim Creek observation area
for 1964 were flooded with one to two and a half feet of
water. Flooded conditions remained from June 21 to August 10,
after which the meadows remained muddy until the middle of
September when further drying permitted the reappearance of
plant growth by the end of the month. There was neither crab
grass nor crickets   present. To test the viability of
eggs deposited in 1964 in these wet meadows, soil samples were
brought into the laboratory in October 1965 from sites where
oviposition had been observed. Eggs were sorted out from the
mud by sieving under water.   All the eggs were found to be
dead and decayed.   Thus there was high mortality of the
previous year's eggs probably as a result of flooding the
oviposition sites for about 51 days. Observations on seas-
onal life history were made in the unflooded edge of the
spruce grove on the northern side of Atim Creek meadows
and in another meadow about two and one half miles away
which dried soon after the heavy rainfall in June. The
seasonal history in 1965 closely approached that of 1964,
as the first singing was heard on August 10.   On this date,
just as in 1964, there were some adults present. However,

5th and 6th instar nymphs predominated.   On September 24-25,
0.9 inches of snow fell which stayed for about a day and a
half. This snow and continuous night frosts from
September 22 to 28 killed most of the crickets in the field.
From this time onwards very few crickets were found. The
last song of the year was heard on October 13, a very clear
sunny day with a temperature of 12.2°C in the afternoon. The
adult population was maximum by the end of August in each
year and started to diminish by September 10.   There was
one generation per year as eggs laid in the summer of one
year hatched in July of the following year.

4.3 The seasonal incidence of diapause

4.31 Introduction

Almost synchronous hatching in July and over-winter-
ing of the egg stage suggest that N. fasciatus possesses a
regulating mechanism by which the seasonal rhythm of devel-
opment is adapted to this climate. This mechanism could be
the changing photoperiod or changing daily temperature during
late summer.

The importance of changing photoperiod in diapause
has been observed by Kogure (1933) in Bombyx mori L.,
Dickson (1949) in Grapholitha molesta (Busck.) and Corbet
(1956) in Anax imperator Leach.

An experiment was conducted to determine whether
the changing photoperiod or some other factor, such as

temperature influenced the females to lay diapausing or non-diapausing eggs.

4.32 Methods and materials

Adults were collected every week in the field from August 7 to October 10, 1964, and kept in battery jars which, after being marked, were put in screen-cages of two cubic feet volume and kept outside under natural conditions of photoperiod and temperature. The locations of the cages were carefully selected to avoid the light from street lights and shadows from buildings and trees. Each jar was provided with food, water and paper towelling as mentioned previously. Wet soil in plastic petri dishes was supplied for oviposition and renewed every 24 hours. Throughout the experiment eggs were sieved out in water and incubated at $29 \pm 1°C$ in petri dishes. In all 4,644 eggs were incubated at this temperature.

Five hundred and fifty eggs in total were incubated separately under different temperature regimes and on different dates. These regimes were as follows: $29 \pm 1°C$, $22 \pm 1°C$, and $20 \pm 1.5°C$. Dates were ranged one week from each other (August 16 to September 24). This was done to observe the influence of different incubation temperatures on incidence of diapause. Temperatures in the cages were recorded with thermographs. Data for Edmonton photoperiods for the complete year was obtained from Dominion Public Weather Office, Edmonton.

4.33   <u>Results</u>

The percentage hatching of eggs deposited during
succeeding weeks of the summer of 1964 and incubated at
29 ± 1°C are shown in Table I.   Table 2 shows the per-
centage of eggs diapausing in relation to fluctuations in
temperature.   Those eggs which hatched in 16 days are
present in Table 1.   The remaining eggs which did not hatch
in this much time were considered as diapausing.   These
results do not show a decisive trend in relation to changing
photoperiod and percentage diapause.   In fact there is no
obvious correlation between the incidence of diapause and
any other weather factor, except for the variation in the
day and night temperatures.   It is interesting to observe
that this fluctuation in temperature which existed at the
time of oviposition was the apparent cause for fluctuations
in the incidence of diapause.   Eggs which were laid during
the weeks with fluctuations between 15.5 to 27.7°C and
between 1.7 or 0.6 to 15.5°C are less diapausing as compared
to those which were laid when the variation was between
below 10 to above 20°C.

The possible explanation to this point is that
either the eggs within the female are directly subjected to
the changes in environmental temperature because crickets
only hide under grass or debris when temperatures are low,
or they are influenced indirectly through some physiological

TABLE I.

A comparison of percentage hatching of eggs deposited by
N. fasciatus under natural conditions of photoperiod during
the summer of 1964, and incubated at 29°C.

Dates of ovipositions	Mean hours of daily light	Percentage Hatchings in 16 days
Aug. 8-14	15 - 12	63.2
Aug. 15-21	14 - 42	27.6
Aug 22-28	14 - 15	24.9
Aug. 29-Sept.4	13 - 47	46.7
Sept. 5-11	13 - 17	34.8
Sept. 12-18	12 - 48	37.0
Sept. 19-25	12 - 19	21.6
Sept. 26-Oct.2	11 - 49	70.7
Oct. 3 -9	11 - 20	40.4
Oct. 10 and 11	11 - 02	71.2

TABLE 2

The percentage of diapausing eggs deposited in succeeding summer weeks by N. fasciatus under natural conditions in relation to the range of temperatures existing for a 48 hour period of oviposition.

Week	Mean temp °C	Temperature Amplitude	Temperature range °C	Percentage of diapausing eggs.
Aug 8-14	20.5	12.2	15.5-27.7	36.8
Aug. 15-21	18.8	16.7	10.0-26.7	72.4
Aug. 22-28	15.0	19.5	7.2-26.7	75.1
Aug. 29-Sept.4	12.2	12.8	7.2-20.0	53.3
Sept.5-11	9.3	24.0	-1.0-23.0	65.2
Sept.12-18	12.8	22.2	3.3-25.5	63.0
Sept.19-25	16.6	22.8	2.2-25.0	78.4
Sept.26-Oct 2	8.8	13.8	1.7-15.5	29.3
Oct. 3-9	12.5	23.8	1.7-25.5	59.6
Oct. 10-11	7.2	14.9	0.6-15.5	28.7

mechanism in the female that is affected by fluctuating temperatures. From the above observations it appears that eggs which remain at fairly high temperatures or fairly low temperatures before their incubation are less diapausing as compared to those which experienced wider fluctuations in temperature. Furthermore, it is apparent from Table 2 that in the first halves of August and October the number of diapausing eggs is less than in September. Thus a second factor contributing to the fluctuations in percentage of diapausing eggs could be the age of females. Diapause in many eggs results in a prolonged hatching period. A typical curve of percentage hatching of total eggs versus incubation days at 29 ± 1°C is illustrated in Fig. 2.

There was heavy oviposition on very warm and particularly on sunny days, especially when such conditions prevailed for two consecutive days. Below 15°C there was very little or no oviposition even in September. It was interesting to note in eggs which were incubated at different temperatures that 35.6 percent hatched in 16 days at 29 ± 1°C. At 22 ± 1°C no hatching occurred within 25 days, 3.5 percent hatched in 60 days and 4.3 percent in 90 days. At 20 ± 1.5°C there was only 3 percent hatching in 90 days. Thus all the eggs incubated at 22 ± 1°C or 20 ± 1.5°C diapaused. It can be concluded, therefore, that higher incubation temperatures suppress diapause.
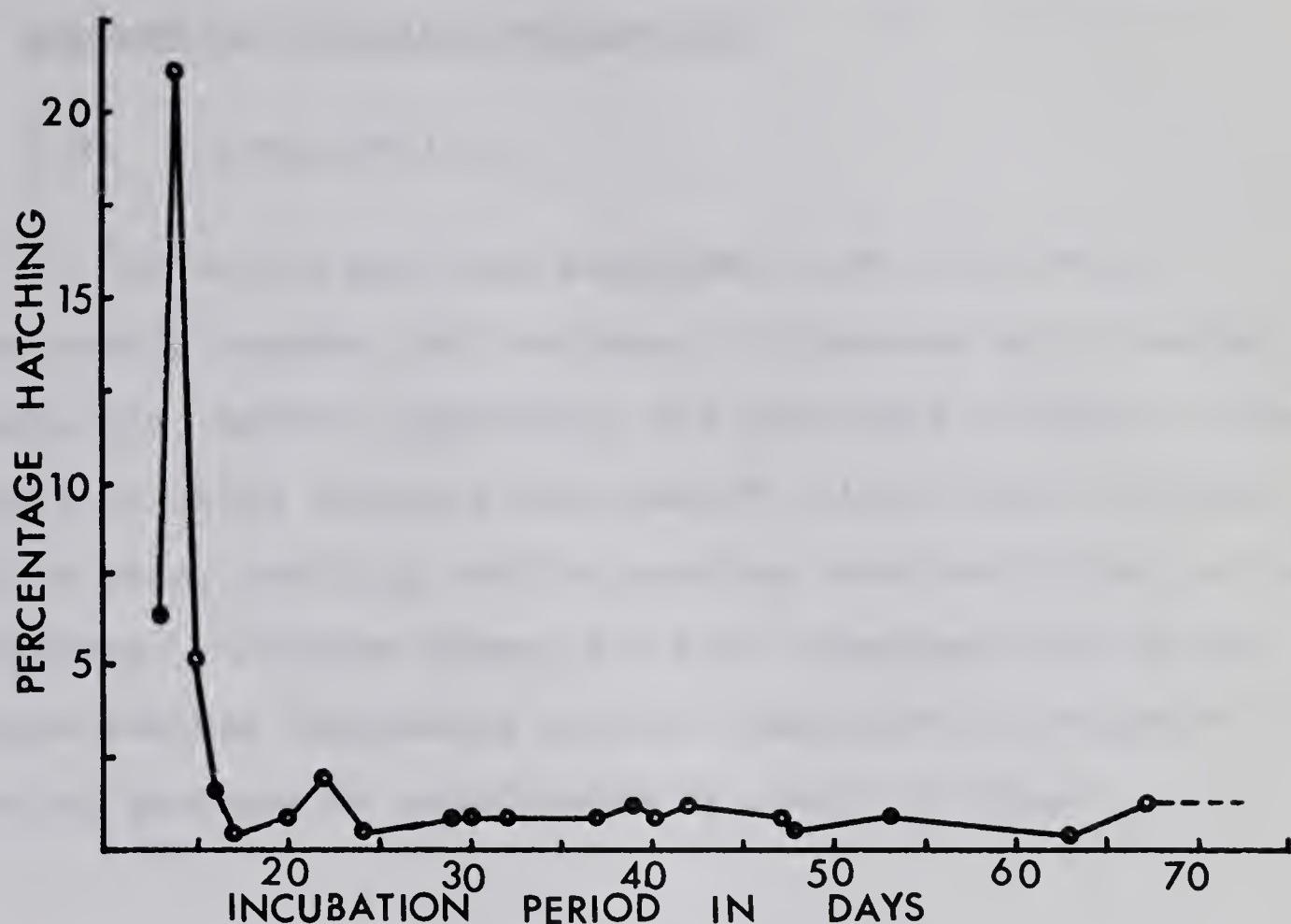
Figure 2. Frequency distribution of hatching of eggs of <u>N. fasciatus</u> at an incubation temperature of 29°C. N = 250.

4.4 The incidence of diapause in eggs deposited by females exposed to changing photoperiod.

4.41   Introduction.

Since the previous experiment did not show any relationship between the incidence of diapause and changing photoperiod, another experiment was conducted to verify those results by using changing photoperiod while other conditions such as food, humidity and temperature were controlled in the laboratory.   Another reason for this experiment was to determine whether diapausing eggs are deposited in response to changing photoperiod experienced by adults or nymphs.

4.42   Methods and material.

Last instar nymphs of both sexes were collected on July 29, 1964 and kept in battery jars in lots of 20 per jar with food, water and paper strips.   These jars were placed in a growth chamber (Sherer, Michigan U.S.A. No. cel 25-7) provided with fluorescent and incandescent lights operated by time switches, and a humidity control.   Two groups of these were exposed to changing photoperiod and the other two served as controls kept in constant darkness. The photoperiod corresponding to September 1 in Edmonton was first used in order to break the continuity of the July photoperiod experienced in nature by the nymphs. A light

intensity of 500 foot candles was recorded on the surface of
the shelf holding the battery jars. The photoperiod was
changed to that of September 6 after five days and similar
changes continued until the photoperiod was that of October 28,
1964. Temperature was maintained in the chamber at 28.9 $\pm$ 0.25°C
for 12 hours per day and at 1.5 $\pm$ 0.25°C for the remain-
ing 12 hours. The relative humidity was maintained at
68 percent. The control battery jars were covered securely
with a light proof cover made of plastic which was black on
the inside and white on the outside. The crickets matured
from July 30 to August 3 and eggs were laid mainly from
August 10 to September 23. They were collected every day
and incubated at 29 $\pm$ 1°C.

4.43 Results.

As shown in Fig. 3 and Table 3 only those eggs which
were deposited by crickets when under 12'36" and 9'49"
photoperiod per day, showed a high percentage hatching
(45.9 percent and 43.3 percent, respectively) whereas 15.5
to 29.2 percent of the rest of the eggs hatched. When
these results were compared with those of field adults laying
eggs under the same photoperiods, there is no similarity.
Changing photoperiod does not have any apparent relation
to the incidence of diapause. Furthermore, the percentage
hatching of eggs laid by the control crickets is significantly

Figure 3. The percentage of diapausing eggs laid under decreasing photoperiod by crickets kept at 28.9°C for 12 hours and 1.5°C for remaining 12 hours per day. Incubation temperature was 29°C.

TABLE 3

A comparison of percentage diapausing eggs of <u>N. fasciatus</u>
deposited in the laboratory under changing photoperiod and
in field cages with corresponding photoperiod.   Incubation
temperature was 29°C.

In laboratory			In field cages
Daily hours of light	Date of oviposition	Percentage diapausing eggs	Percentage dia-pausing eggs laid on a date corres-ponding to that in the laboratory with same photo-period.
12-36″	Aug. 10	54.0	75.7
11-53″	Aug 24	70.8	62.0
11-13″	Sept. 3	78.5	51.4
10-54″	Sept. 8	75.9	
10-20″	Sept. 13	84.5	
9-49″	Sept. 23	56.7	

lower than that of illuminated ones (Table 4). This could, however, result from the absorption of radiant heat by the illuminated crickets.

It was also observed in the control groups that there was no significant difference in percentage hatching in eggs deposited by females of different ages since the Chi-square for independence (X^2=14.22; 5%=18.31 and 1%=23.21) is not significant. From Fig. 4, however, it seems that the incidence of diapause in eggs laid by young and old females is less than in those laid by middleaged crickets.

4.5 Nymphal development at constant temperatures

4.51 Introduction

There is no information concerning the number and length of nymphal stadia in N. fasciatus under normal conditions or in the laboratory. Nymphal development was studied in the laboratory to permit recognition of stages collected from the field at different times. Moreover, information on the length of nymphal stadia was required in the experiment in which different instars were given photoperiod treatments.

4.52 Methods and material

Eggs were collected from females kept in the lab-

TABLE 4

The percentage of diapausing eggs of <u>N. fasciatus</u> deposited by illuminated females and those which were kept in the dark (control). Incubation temperature was 29°C.

Illuminated Crickets		Control Crickets	
Total number of eggs	Percentage diapausing	Total number of eggs	Percentage diapausing
362	76.80	357	87.1

x^2 for independence = 13.84**

5% probability level = 3.84

1% probability level = 6.63
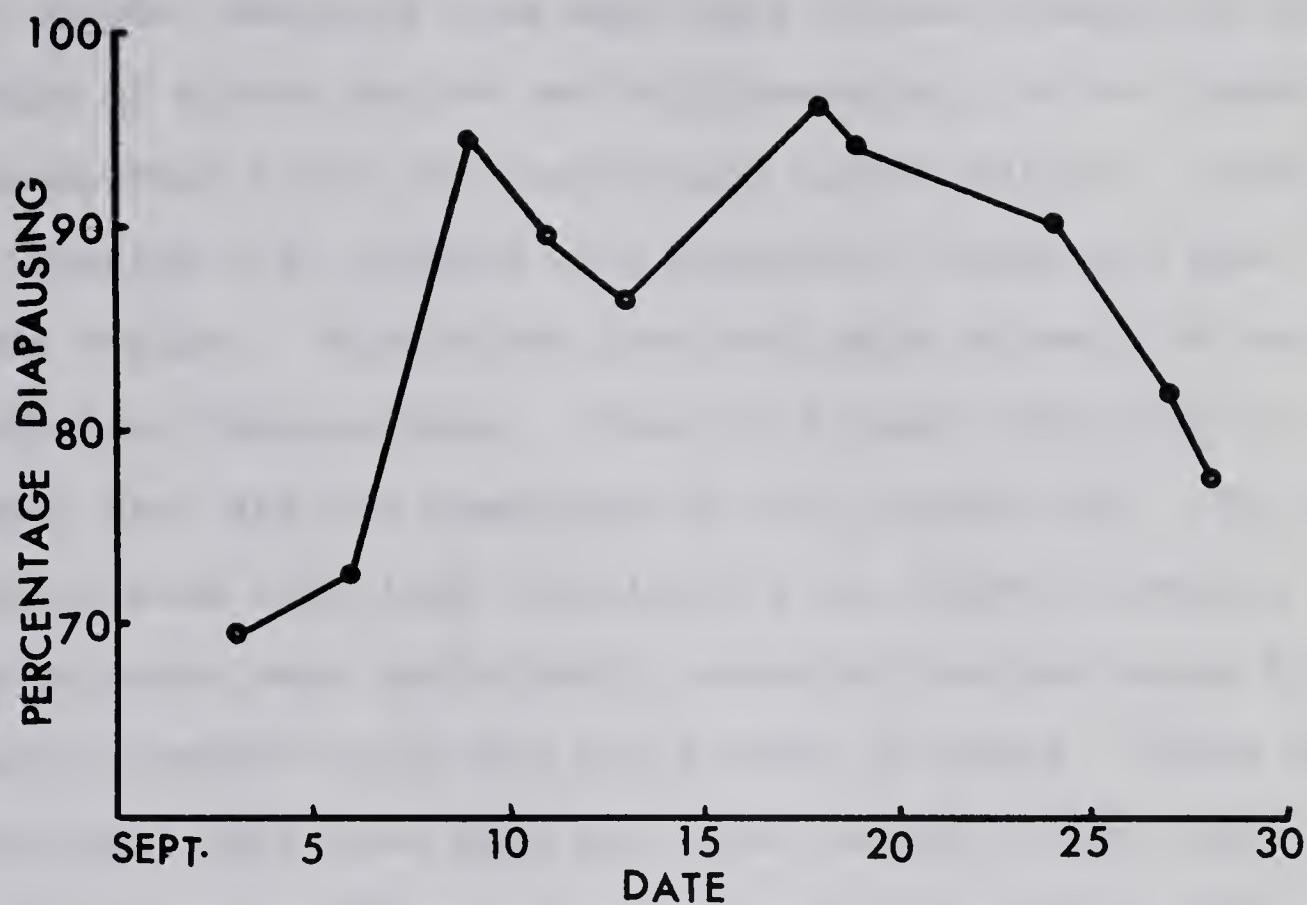
Figure 4. The percentage of diapausing eggs laid on succeeding dates by crickets kept in darkness at 28.9°C for 12 hours and 1.5°C for remaining 12 hours per day. Incubation temperature was 29°C.

oratory. They were maintained at 5 ± 1°C for 30 days to break diapause and then incubated at 29 ± 1°C or 24 ± 1°C. All nymphs emerging from eggs were divided every day into groups of either ten or two which were kept in one pound wide-mouthed fruit jars containing rabbit pellets, water in an inverted vial plugged with absorbent cotton and some paper strips. Forty-five jars were kept at each of the incubation temperatures. Twenty of these contained 10 nymphs each and the remaining 25, two nymphs each. The jars were covered with lids containing fine insect screening and the crickets were individually observed for moultings by removing paper strips and vials every 24 hours. These observations continued from the first instar to the adult stage. For the first three nymphal stadia, the light color of newly moulted nymphs, and body size (in particular the width of the head capsule between the eyes) were the criteria used to determine new moultings. Exuviae were of little value as they were usually consumed by the nymphs. Nymphs older than the fourth instar were examined for the length of wing pads and ovipositor. Measurements were made with an ocular micrometer.

4.53   Results

There are six nymphal stadia. The mean duration (in days) of each nymphal stadium at two temperatures is set

TABLE 5

The mean duration of nymphal instars of <u>N.</u> <u>fasciatus</u> in days at 24° and 29°C.

Instar	Temperature			
	24°		29°C	
	Mean	S.D.	Mean	S.D.
1st	5.8	0.38	4.1	0.47
2nd	5.5	0.52	3.8	0.66
3rd	5.7	0.61	4.0	0.80
4th	6.3	0.71	4.4	0.90
5th	8.0	1.03	5.2	0.82
6th	9.8	1.03	6.6	0.74

TABLE 6

The head width in millimeters between the compound eyes of different nymphal instars of <u>N. fasciatus</u> reared in the laboratory.

Stage	Mean	S.D.
1st instar	0.5	0.02
2nd instar	0.6	0.02
3rd instar	0.7	0.05
4th instar	0.9	0.02
5th instar	1.0	0.07
6th instar	1.2	0.05
Adult	1.4	0.09

out in Table 5, whereas Table 6 contains the mean width of
the head capsule between the compound eyes.   The first three
nymphal stadia are almost of the same duration at each of
the two temperatures, whereas, the fourth, fifth and sixth
stadia differ considerably in their durations.   There is a
difference of about 0.1 mm. in the width of the head cap-
sule between the first three instars.   Only injured and
dying nymphs were attacked and eaten by healthy ones. . Other-
wise no cannabilism was observed in either size of group.

4.6   Effect of submergence of eggs in water.

4.61   Introduction

It was observed previously that two to three days
exposure to muddy soil was not detrimental to eggs, while
51 days was. The effect of submergence of eggs in water
in the laboratory was, therefore, studied.

4.62   Methods and materials

Eggs were obtained from females which were collected
from Atim Creek meadow, in 1964 and which oviposited on
September 27 and 28. Four hundred and fifty eggs were kept
at 5 ± 1°C from the end of October 1964 to July 5, 1965 when
the following experiment was started.   Five groups each of

75 eggs were treated by submerging them under tap water in bottles for 5, 10, 15, 20, and 25 days. A remaining group of 75 eggs was incubated in the normal manner to serve as a control. Each bottle was provided with one inch of wet soil. Eggs were placed a few millimeters below the surface of the soil with the help of a pair of forceps and a brush. The bottles were then filled with tap water gently without disturbing the eggs and kept at 29 $\pm$ 1°C. After treatment eggs were sieved out and incubated at 29 $\pm$ 1°C.

4.63   Results

Table 7 shows the percentage mortality and the minimum incubation period after immersion. Eggs immersed for five days started hatching after five further days of incubation, whereas in the control the minimum incubation period was 10 days. After 10, 15, 20 and 25 days immersion a further minimum incubation period of two days was required. Thus, development continued at about its normal rate at 29 $\pm$ 1°C under water for eight to nine days and then ceased. For five days submergence there was 27.0 percent mortality as compared to 15 percent in the control. There is a significant correlation at the one percent probability level between duration of submergence and percentage mortality. These results show that after five days, submergence of eggs in water is detrimental and about three-quarters of the eggs

TABLE 7

The lethal effect of submergence in water for different

periods on eggs of <u>N. fasciatus</u>.

Days of Immersion	Minimum incubation period in days.	Percentage Mortality
0	10	15.0
5	5	27.0
10	2	32.0
15	2	39.0
20	2	57.0
25	2	72.0

γ_{xy} (correlation coefficient) between period

    of submergence and percentage mortality   = 0.97**

              5% probability level   = 0.88

              1% probability level   = 0.95

in the field will be killed in 25 days of flooding.

4.7 Discussion

Differences in the dates of the first and last singing in Ohio, Michigan, and Edmonton seem to be due to the difference in temperatures at these places. North Carolina is warmer than Ohio and Michigan, and two to three generations per year are possible (Fulton, 1931). In Florida the temperature remains fairly high throughout the year and the variation in the mean monthly temperatures for the years 1941-50 (World weather records, 1959), is only between $11.4°$ to $27.7°C$, which allows this cricket to breed throughout the year. Fig. 5 shows a comparison of mean monthly temperatures at four localities with the type of life cycle at some of these places. It appears that the number of generations per year at different latitudes is influenced by the period of high temperature available.

Since the mean maximum temperature in Edmonton during August, September and October rarely goes above $22.5°C$ there is no hatching of eggs deposited earlier in the season which is in accordance with the laboratory re-sults (only 4.3 percent hatch occurred in 90 days at $22 \pm 1°C$). A few nymphs which were seen in the late summer probably hatched late from the previous year's eggs or were exceptionally slow in developing. This point was
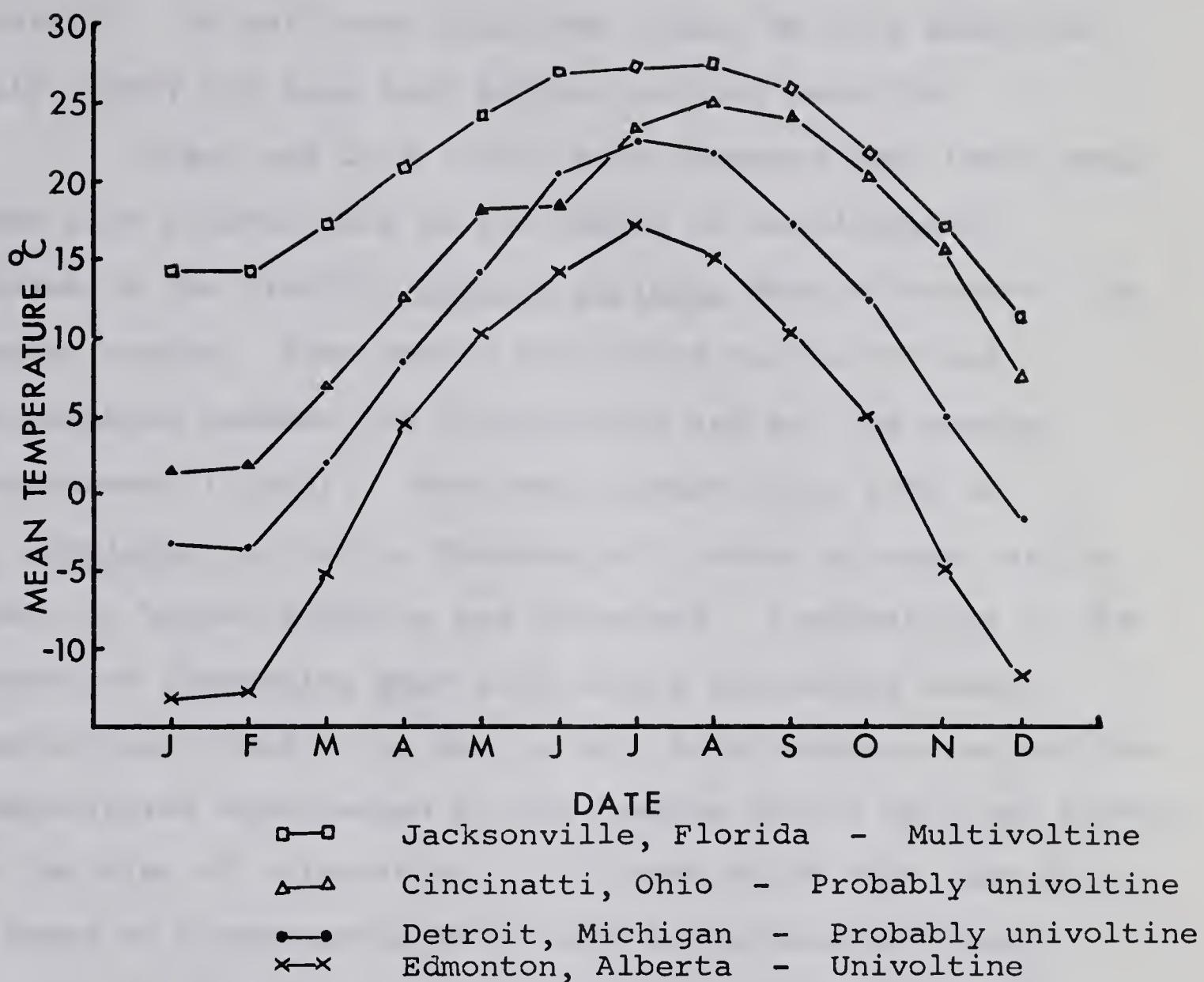
Figure 5. Mean monthly temperatures (C°) at

different localities in relation to voltinism

in <u>N. fasciatus</u>.

cleared up during the study of the life history in the laboratory. It was found that some nymphs develop exceptionally slowly and some died before reaching maturity.

Cragg and Cole (1952) have observed that there were very high fluctuations in the number of non-diapausing larvae in the blowfly, _Lucilia sericata_ Meig. throughout the summer months. They stated that there was no obvious correlation between the fluctuations and any one weather measurement (p.603). The same appears to be true of _N. fasciatus_, so far as photoperiod, number of eggs laid or hours of bright sunshine are concerned. Fluctuations in the number of diapausing eggs laid during succeeding summer months were found to be due to both high temperatures and low temperatures experienced by the females during days and nights at the time of oviposition. Crickets which were exposed to a range of temperatures which were favourable for embryogenesis (15.5 to 27.7°C), and temperatures (0.6 to 15.5°C), which were favourable for breaking diapause, laid less diapausing eggs. A large number of diapausing eggs were laid by the crickets exposed to widely fluctuating temperatures (below 10 to above 20°C).

Many articles have been published which deal with the effect of seasonal fluctuations in temperatures on the induction and termination of diapause. At present it is a generally accepted principle that diapause termination in

most insects needs low temperatures whereas embryogenesis in post-diapause stages requires high temperatures to proceed. There has been, however, very little work done on the effects of daily fluctuations of temperature on diapause. Kozhanchikov (1949) associated diapause in the Chinese oak silkworm _Antheraea pernyi_ Guer. with the effects of daily fluctuations in temperature on the larvae. He found that all of the larvae transformed into non-diapausing pupae when the daily temperature varied from 19 to 22°C at a mean rearing temperature of 20°C. However, with a range of 18 to 25°C at the same rearing temperature the majority (86 percent) of the larvae diapaused and all the larvae diapaused when the daily fluctuations in temperature varied from 18 to 32°C, 15 to 30°C and 15 to 32°C. At a slightly lower rearing temperature (17°C) all larvae underwent diapause when the daily temperatures varied from 15.2 to 25.0°C, 14.0 to 25.0°C and 14.0 to 30.0°C. Therefore, in _Antheraea pernyi_ Guer. both the mean rearing temperature and the range of fluctuations are important in invoking diapause.

Browning (1952b) observed in _Acheta commodus_ Walk. (formerly _Gryllulus commodus_) that as the incubation temperature is raised, an increasing percentage of the eggs develop without diapause. His observations are confirmed by Hogan (1960a). This also holds good in _N. fasciatus_, as is evident from percentage hatch at 29 ± 1°C, 22 ± 1°C and 20 ± 1.5°C.

Harmful effect of submergence of <u>N. fasciatus</u> eggs in water was manifested after five days at 29°C. Barber and Dicke (1939) have also shown in <u>Heliothis armigera</u> Hubner, that pupae are killed by water; moreover, mortality increases with a rise of temperature. At 23.9°C 20 percent of the pupae survived submergence for 10 days, in contrast to 84 percent at 4.4°C.

From field observation and these experiments it seems that this species is univoltine in Edmonton and embryogenesis is controlled by temperature rather than photoperiod. Non-diapausing eggs cannot hatch the same year, firstly because it is too cold and secondly the big differences between day and night temperatures induce diapause.

5.   EFFECTS OF LOW TEMPERATURES ON DIAPAUSE

5.1 Introduction

Diapause is the chief physiological mechanism for
the seasonal regulation of development in insects of temp-
erate regions (Andrewartha, 1952;   Lees, 1955;   Danilyevsky,
1965; DeWilde, 1962).   While both temperature and photo-
period are the main environmental factors which invoke and
terminate diapause, Lees (1955) stated that temperature is
by far the most important environmental agency controlling
the termination of the diapause (p.53).   There are many
examples of insects from several orders in which diapause
has been broken by exposure of the diapausing stage to low
temperatures.   It has been shown in a number of cases in
Orthoptera (Parker, 1930: Burdick, 1937;   Andrewartha,
1943;   Church and Salt, 1952;   Browning, 1952a,b;   Hogan,
1960a,b;   Rakshpal, 1962a,b;   Masaki, 1962) that exposure
of the diapausing eggs to low temperatures for an optimum
period of time causes them to develop readily when incub-
ated at an appropriately higher temperature.

The effects of continuous low temperatures for
different periods and alternating low and high temperatures
on diapausing and post-diapausing eggs were investigated.
In discussing the results of these experiments I have
arbitrarily described hatching within three days

of first hatch of up to 30 percent eggs as indicating no
effect on diapause and above 80 percent as indicating full
breaking of diapause.   Hatching between 30 and 80 percent
is described as partial breakage of diapause.

5.2 Effects of low temperature on eggs of different ages.

5.21   Introduction

It was found from field observations that eggs which
were laid during different summer months were subjected to
low temperatures after remaining at high temperatures for
varying periods of time, that is, eggs laid in August and
September undergo a longer period of high temperatures as
compared to those which were laid in October. It has been
shown in some orthopteran species (Parker, 1930; Church and
Salt, 1952; Browning, 1952a,b) that diapause is broken in
eggs which are exposed to low temperature before the diapause
stage is reached. But until recently the effect of low
temperatures on eggs of different ages has scarcely been
explored, except for some work by Hogan (1960b) on
Acheta commodus Walk., Rakshpal (1962b) on Gryllus pennsylvanicus
Burm. and Masaki (1962) on the Emma field cricket
Gryllulus mitratus (Burm.).

An experiment was carried out primarily to determine
the relationship between low temperatures and the breaking of

diapause in eggs of various ages. However, this was also done to see if low temperatures had any detrimental effect on these eggs. Rakshpal (1962b) observed very high mortality when one day old eggs of Gryllus pennsylvanicus Burm. were exposed to low temperature.

5.22 Methods and materials

About 80 crickets were collected from the field and kept in the laboratory in four battery jars, with food, water and paper towels. Fourteen hundred eggs were collected on each of two alternating days from eggs laid within 24 hours. Each group of fourteen hundred eggs was divided into lots of 50. Two replicates each of 50 eggs were moved in each case after keeping at 22 $\pm$ 1°C for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 16 and 21 days (referred later as the initial incubation period) to either 0 $\pm$ 1°C or 5 $\pm$ 1°C temperatures for 30 days. Thereafter these eggs were incubated at 29 $\pm$ 1°C. The zero day initial incubation meant that eggs which were laid within 24 hours were exposed directly to low temperatures, whereas one day initial incubation meant that eggs laid within 24 hours were kept at 22 $\pm$ 1°C for one day and so on.

5.23 Results

Table 8 shows that eggs which were given zero day

TABLE 8

The effect of exposure to low temperature on termination of diapause and mortality in eggs of <u>N. fasciatus</u> kept at 22°C for different periods after being deposited. Incubation temperature was 29°C.

Days of initial incubation at 22°C	0°C			5°C		
	Mean incubation period in days at 29°C	Percent Hatching	Percent Mortality	Mean incubation period in days at 29°C	Percent Hatching	Percent Mortality
0	14.0	52[1]	33	13.5	89	10
1	13.5	91	7	12.9	89	5
2	12.6	95	3	12.4	90	3
3	12.0	98	2	11.8	95	3
4	11.3	97	2	11.4	94	1
5	10.8	94	5	11.1	95	4
6	10.6	93	5	11.3	93	4
7	10.0	87	11	10.0	94	0
8	9.9	96	3	10.0	97	0
9	9.4	96	2	9.9	94	1
10	9.3	96	4	9.3	98	2
11	9.8	92	3	9.4	86	5
16	10.7	74	4	10.1	86	3
21	11.5	62	8	10.6	76	6

1.   Some eggs hatched after very long periods.

'F' value for different initial incubation periods   = 3.13*
   5% probability level   = 2.60
   1% probability level   = 3.96

'F' value for interaction of low temperatures and initial incubation periods   = 4.49**
   5% probability level   = 2.13
   1% probability level   = 2.93

initial incubation and exposed to 0 ± 1°C showed higher
mortality (33 percent) than those which were kept at
5 ± 1°C (10 percent).   The percentage mortality was not
much different for the rest of the eggs.   There was no
water uptake in eggs during their exposure to low temper-
atures after incubated initially for zero to three days.
In these eggs water uptake occurred, eventually, during final
incubation at 29 ± 1°C within seven days.   In eggs
which were given an initial incubation of four to six days,
only some completed water uptake before being exposed to
low temperatures.   After a seven day initial incubation
period all eggs were fully swollen with water.   Thus water
absorption takes place only at incubation temperatures.
Hatching, in eggs without initial incubation and for both
low temperature treatments started on the 13th day and
continued mainly up to the 18th day of incubation.   In these
eggs maximum hatching occurred, however, on the 14th day of
incubation.   In all the eggs which were incubated initially
for 2 to 11 days, hatching occurred mainly within a range
of two to three days;   for the longer initial incubation,
however, this range was extended to a period of six to seven
days.   The period of maximum hatching decreased from 14 to
10 days as the initial incubation increased from zero to
seven days.   It seems from this difference of four days in
these incubation periods that eggs without low temperature

treatment developed at 22 ± 1°C in seven days to the stage
they had developed at 29 ± 1°C in four days.   Thus the
total incubation period at 29 ± 1°C for all the post-dia-
pausing eggs is almost always 13 to 15 days.   For more than
seven days initial incubation maximum hatching took place
on the 10th day (sometimes on the 9th day) of final incub-
ation.   Zero day initial incubation caused a lower percent-
age hatching due to higher mortality in these eggs.   Eggs
which were incubated initially for 16 and 21 days showed a
somewhat lower total percentage hatching as compared to those
which were given 1 to 11 days initial incubation.   From this
it follows that eggs which were kept at 22 ± 1°C for longer
periods before chilling might have undergone diapause more
strongly as compared to eggs which were exposed to low temp-
erature earlier.   This possibility is supported by a compar-
atively wide range of hatching (six to seven days) in the
former eggs.   This wide range of hatching period also causes
higher mean incubation period for these eggs.   They, there-
fore, might require longer low temperature treatment to
break diapause.

It was found by analysis of variance that the variation
in percent hatching among treatments is significant at the one
percent probability level due to interaction of low tempera-
tures and initial incubation periods.   Variation in percent-
age hatching among treatments of initial incubation periods,
however, is only significant at the five percent level.

5.3   Effects of low temperatures on breaking diapause.

5.31   Introduction

N. fasciatus is found in extensive areas of North
America where temperatures differ widely in winter, from
which it follows that this cricket possesses some mechanism
by which diapause is terminated by the available local low
temperatures.   Lees (1955) found that diapause termination
in insects from warmer localities does not require very low
temperatures as compared to that in insects inhabiting colder
environments.   Danilyevsky (1965) and Masaki (1961) think
that genetical differentiation for terminating diapause by
local low temperature is often involved in the case of insects
with a seasonal rhythm of development adapted to different
climatic areas. Bigelow (1960, 1962) reported variations
in adaptability to local low temperatures in local popul-
ations of field crickets inhabiting different localities in
North America.

I was interested, therefore, in determining the range
and periods of low temperatures effective in breaking dia-
pause.

5.32   Methods and materials

Eight hundred eggs, which had been laid over a 24
hour period during September, 1964, were divided into 16 groups

of 50.   All groups were incubated initially at 22 ± 1°C
for 10 days. By this time all had reached the diapause stage,
thus less mortality was expected.   Four groups each were
then kept in rooms maintained at the following temperatures:
0 ± 1°C, 5 ± 1°C, 10 ± 1°C, and 15 ± 1°C.   After 15, 30, 45
and 60 days one group of eggs from each room was incubated
at 29 ± 1°C.

The same procedure under similar conditions was re-
peated in 1965 to replicate the experiment.

5.33   Results

The percentage hatching in 11 days (the normal in-
cubation period for post-diapause eggs incubated previously
for 10 days) against time of exposure to low temperatures is
plotted in Fig. 6.   All four temperatures broke diapause
quite effectively. Fifteen days exposure to low temperature
had only a partial influence on breaking diapause since the
percentage hatching was 60 to 72 .   The percentage hatch-
ing for 30 days exposure treatments ranged from 78   - 94 .
Forty-five day exposures broke diapause almost completely.
Sixty days low temperature treatment enabled virtually all
of the eggs to develop readily at 29 ± 1°C. Frequency
distributions of the percentage hatching of eggs kept for
15, 30, 45 and 60 days at 5°C (Fig. 7) show that the range
of hatching period decreases with increase in time of low
temperature treatment.   It was found by analysis of variance
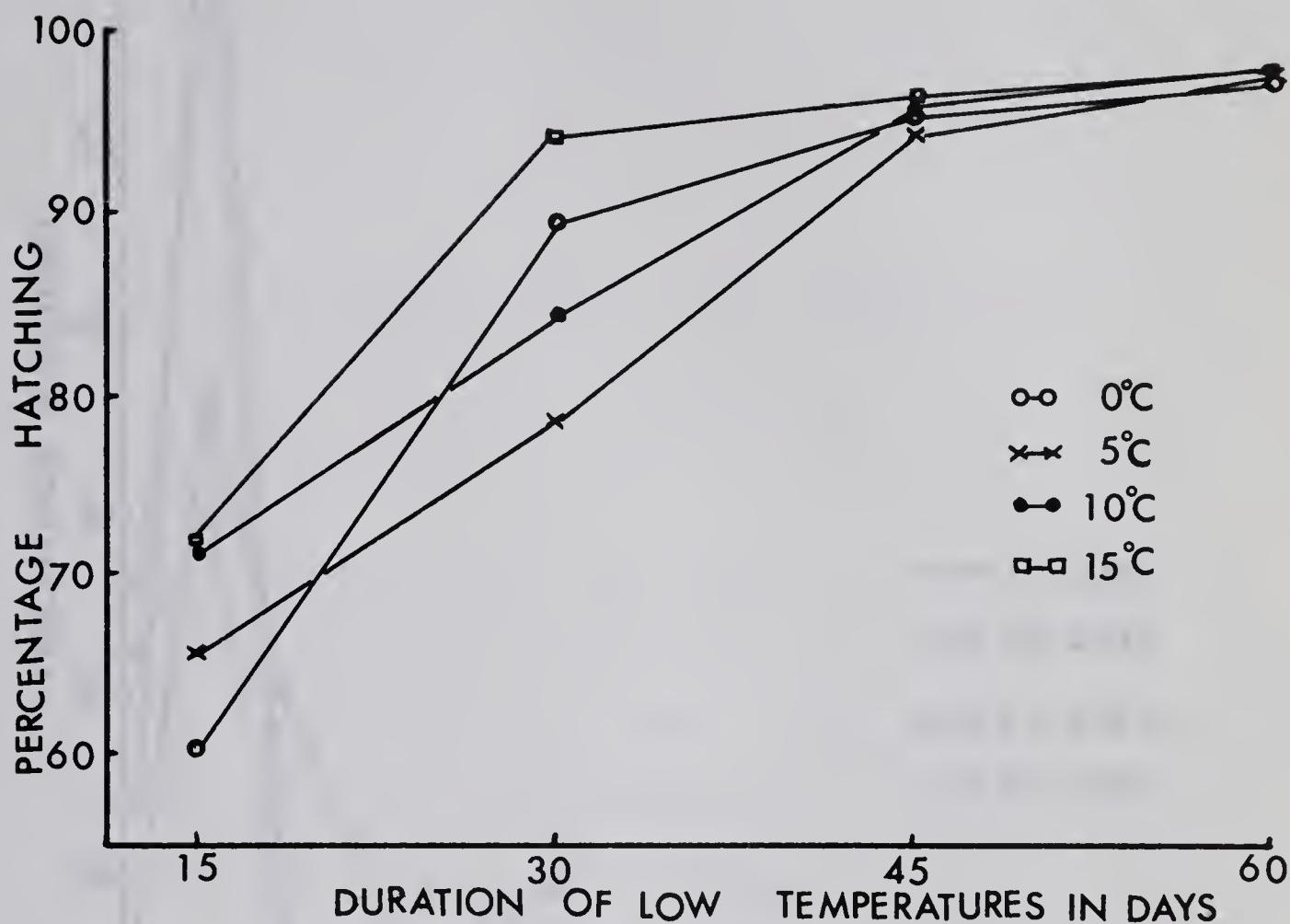
Figure 6. The percentage hatching of eggs of
N. fasciatus which were exposed to different low
temperatures for different periods, prior to
incubation at 29°C.
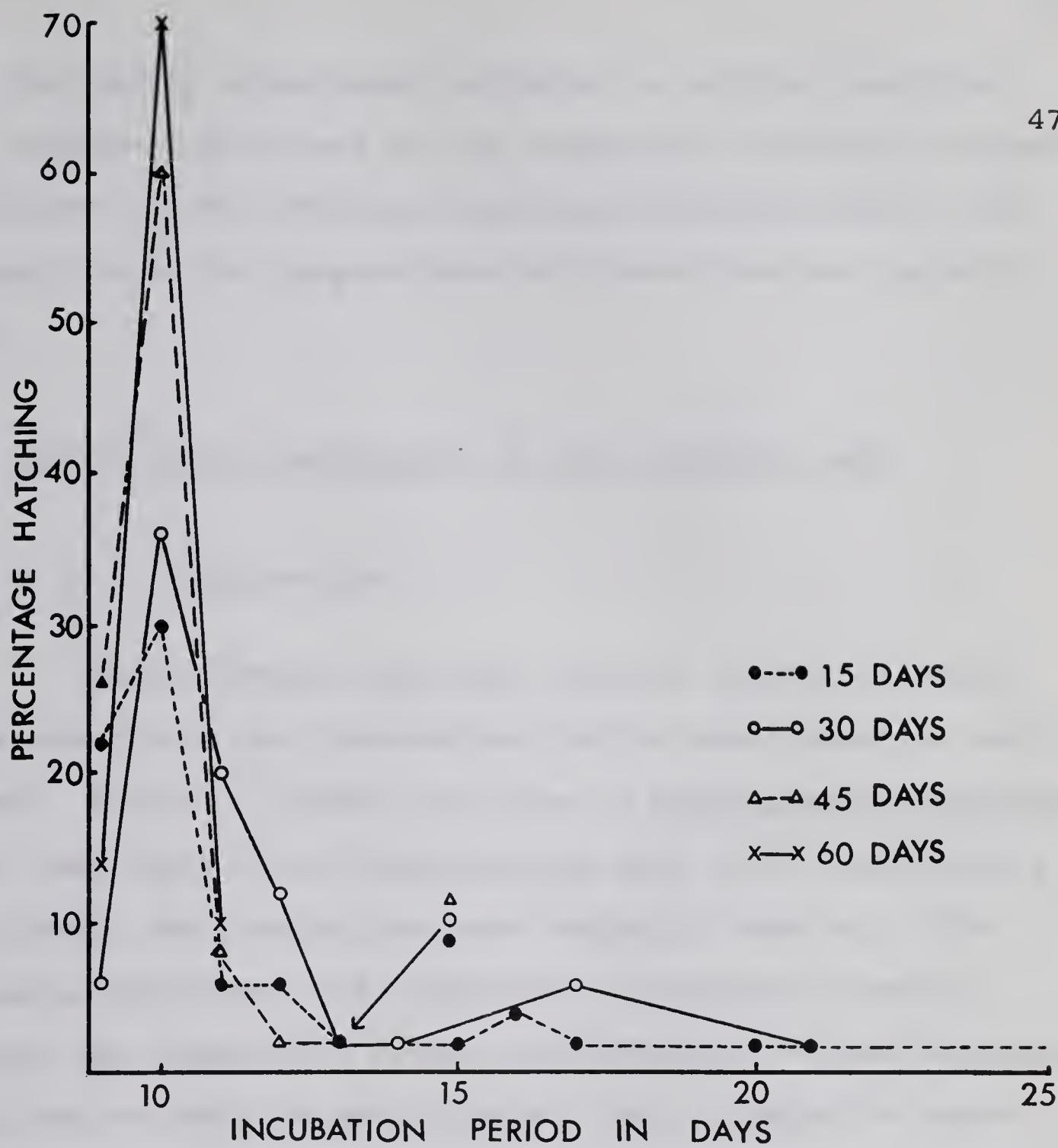
Figure 7. Frequency distribtuion of hatching
of eggs of N. fasciatus exposed to 5°C for 15,
30, 45 and 60 days and then incubated at 29°C.

that the highly significant variation in hatching resulted
from different durations of low temperature treatment, whereas,
variations due to different low temperatures and due to the
interaction of low temperatures with durations are insignif-
icant.

5.4   Effect of low temperature on post-diapause eggs.

   5.41   Introduction

        Post-diapause eggs under natural conditions some-
times experience low temperatures during development in early
summer. Rakshpal (1962b) has shown in Gryllus pennsylvanicus
Burm. that exposure of post-diapause eggs to low temperature
is slightly detrimental, as some mortality occurred. The
following experiment was conducted to determine primarily
whether low temperature causes any mortality in post-diapause
eggs; and secondly to determine how long it takes for eggs
to recover from this chilling to develop normally at incub-
ation temperature.

   5.42   Methods and materials

        Six hundred eggs were counted from eggs which had
been deposited within 24 hours.   Two days later another simi-
lar sample of eggs was obtained for replication.   Each of
these samples was divided into 12 groups of 50.   All the eggs

were left at 22 ± 1°C for 10 days and then transferred to
5 ± 1°C for 30 days to terminate diapause, then brought to
29 ± 1°C.   Three groups (from each replicate) were then in-
cubated continuously at 29 ± 1°C to serve as controls,
whereas, the other nine were moved to 5 ± 1°C (second chilling)
three at a time, after 4, 6 and 8 days of incubation (referred
to later as first post-diapause incubation).   From each of
these three groups one was brought to 29 ± 1°C for a second
or final post-diapause incubation after 5, 10 and 15 days
of second chilling.

5.43   Results

Table 9 shows that the percentage hatching at
29 ± 1°C in all the treatments varied from 80 - 96. Hatching
was greater in eggs which were given six days first post-
diapause incubation and five days second exposure to low
temperature (96 percent hatching).   The control eggs started
hatching on the ninth day of incubation and continued mainly
up to the 11th day (normal incubation period for eggs exposed
to low temperature after 10 days of pre-diapause incubation
at 22 ± 1°C) with maximum hatching on the 10th day.   Eggs
which were incubated at 29 ± 1°C for first post-diapause in-
cubation for 4, 6 and 8 days (regardless of duration of
second chilling) started hatching in their second post-dia-
pause incubation after 5, 3 and 1 days with maximum hatching

TABLE 9

The effect of exposure to low temperature (5°C) on the percentage hatching in post-diapause eggs of N. fasciatus. Incubation temperature was 29°C

Duration of first post-diapause in-cubation at 29°C in days	Duration of second low temperature exposure in days			
	0	5	10	15
	Percentage Hatching			
4	90	92	88	86
6	94	96	91	84
8	87	92	85	80

on the 6th,4th and 2nd day, respectively. It is, therefore, clear that all the groups of eggs have the same number of days of total incubation period after the termination of diapause regardless of the time at which they were exposed to low temperature for the second time and the duration of that time. Thus, once diapause is terminated the development continues at a favourable incubation temperature and the incubation period depends upon the period for which the incubation temperature is available. The presence of low temperatures during development would not harm eggs except for decreasing the rate of development. It was also observed that after the second chilling eggs recovered and resumed their normal development within 24 hours at $29 \pm 1°C$. Statistically there is no significant variation in percentage hatching among all the treatments (including the control), which shows that low temperature has no detrimental effect on post-diapause eggs.

5.5 <u>The effect of alternating high and low temperatures</u>
<u>on induction and termination of diapause</u>.

5.51 Introduction

Since eggs experience low temperature at night and high temperature during day in September and October in the Edmonton region, alternating temperatures might, therefore,

have some bearing on egg diapause in N. fasciatus. The diapause-inducing influence of widely fluctuating daily temperature experienced by the females has already been determined in section 4. The objectives of the present experiment were to determine the effects of alternating high and low temperatures on the induction or termination of diapause in eggs.

5.52 Methods and materials

The high and low temperatures used in the experiment were 29 $\pm$ 1°C and 5 $\pm$ 1°C, respectively. Five hundred eggs deposited on September 13 plus another sample of 500 laid on September 16 to replicate the experiment, were used. Each sample was divided into five groups of 100 eggs. Four such groups from each replicate were exposed to 2, 4, 8 and 12 day cycles of high and low temperatures over a total period of 48 days. Each cycle was formed of two equal durations, one at a low and the other at a high temperature. The two day cycle of alternating high and low temperature treatment would mean one day at high and one day at low temperature alternatively for 48 days. The remaining fifth group of eggs was exposed continuously to low temperature for 24 days to serve as a control. Twenty-four days was the time period chosen, because, for all the treatments the total number of days spent by the eggs at low temperature

was 24. After treatments eggs were incubated continuously at 29 $\pm$ 1°C.

5.53   Results

Table 10 shows that the percentage hatching at high temperature during the treatments increased from 0 to 70 with the increase of 2 to 12 days in the duration per cycle. Some hatching occurred during the treatments because eggs remained at high temperature for a total of more than 16 days, the normal period for incubation. Furthermore, eggs which were subjected to two or four day cycles of high and low temperatures showed a very low percentage hatching when in- cubated continuously at the completion of treatments. In the control, however, 82 percent of the eggs hatched, which is the same as for eggs which experienced six days high and six days low temperature per cycle. An analysis of variance revealed that variation in percentage hatching among different treatments was found to be highly significant. The total percentage hatching for different treatments shows that eggs which were exposed alternatively for one day to high and one day to low temperature were almost all dia- pausing at the end of treatment. Cycles longer than four days are not as effective in inducing diapause. Induction of diapause by fluctuations of low and high temperatures after short periods is a characteristic of N. fasciatus.

TABLE 10

The mean percentage hatching of eggs of <u>N. fasciatus</u> during
and at the end of alternating 29°C and 5°C temperature
treatments for 48 days.

Duration (days) in each cycle	Percent hatching during treatment	Percent hatching after treatment	Total per-centage Hatching
2	0.00	5.00	5.00
4	4.50	16.50	21.00
8	46.50	8.50	55.00
12	70.00	12.00	82.00

Control Percentage hatching   =   82.00

'F' value for treatments   =   62.96**

    5% probability level   =   9.28

    1% probability level   =   29.46

5.6 Discussion

Diapause in N. fasciatus is broken effectively by low temperature even in freshly laid eggs, except that there was a higher mortality as compared to those which were kept at high temperature for more than one day before exposure to low temperature. Lees (1955) mentioned that in many insects, particularly Orthoptera, exposure to low temperature should synchronize with some definite stage in the morphological or physiological development of the embryo to break diapause. He called this stage the period of sensitivity. In N. fasciatus this period extends from zero to seven days at 22 ± 1°C. The stages of embryogenesis in this period are all the stages up to the end of anatrepsis, which were observed by studying 40 eggs after every 24 hours from a sample of 1,000 kept at 22 ± 1°C. It was found from these observations that eggs developed readily without exposure to low temperature up to the completion of anatrepsis (which comes mostly after seven days of incubation at 22 ± 1°C), after which development was blocked in most of the eggs. Rakshpal (1962b) observed an almost similar period of sensitivity in Gryllus pennsylvanicus Burm. .

In N. fasciatus, low temperature is, therefore, effective in breaking diapause before the actual stage of diapause (anatrepsis) is reached. There are other orthopterans described, in which low temperature is effective in

averting diapause before the diapause stage. It has been noted by Parker (1930) in _Melanoplus mexicanus_ (Sauss.); Church and Salt (1952) in _Melanoplus bivittatus_ (Say); and Browning (1952a,b) in _Acheta commodus_ Walk. that eggs which have not reached the diapause stage but which were exposed to an adequate low temperature for an adequate period and then incubated, hatched without diapause. Browning (1952b) found that _Acheta commodus_ is unusual in this respect, because in most species undergoing diapause low temperature is most effective in terminating diapause after the insects have entered diapause.

Hogan (1960a) criticized the observations of Browning from his own experiments on _Acheta commodus_ but confirmed them later (1960b) by stating that exposure of pre-diapause eggs to a temperature of about 13°C so weakens the tendency of eggs to enter diapause that it is readily averted when they are transferred to a suitable incubation temperature (p.528). In fact, in his experiments from which he criticized Browning a suitable incubation temperature was not provided for the eggs after low temperature treatment.

Only a small proportion of the pre-diapause eggs of _Gryllus pennsylvanicus_ Burm. were rendered free from diapause by low temperature (5 - 7°C) and there was such a high mortality that the eggs which were incubated for one day only at 22 - 23°C before exposure to low temperature were completely killed by the latter (Rakshpal, 1962b).

The observation that eggs of N. fasciatus have pre-sumably entered diapause more strongly when kept for longer periods at high temperature before low temperature treatment agrees to some extent with that of Browning (1952a) in Acheta commodus Walk.   He has shown that a preliminary high temperature treatment for more than two days caused the eggs of Acheta commodus Walk. to enter diapause more firmly than if kept for a little or no time at high temperature before low temperature treatment.   He believed that diapause in eggs given a lengthy initial incubation at high temperatures was more intense and so required a longer period for its breaking.   Moroga (1951, cited in Lees, 1955) has also observed in Bombyx mori L. that if eggs were given an initial incubation period ranging from 3 to 80 days, before 40 days of chilling at 5°C, the percentage of non-diapausing eggs dropped from 84 to 8.

Masaki (1962) exposed eggs of the Emma field cricket, Gryllulus mitratus (Burm.) to a high temperature (30°C) for different periods and then kept them at a low temperature (20°C) to determine the effect of duration of high tempera-ture on diapause intensity.   He found that eggs were most responsive to the diapause - intensifying action by 9 or 14 day high temperature treatment when they were in the late pre-diapause and early diapause stages.   His findings, how-ever, were based on the mean incubation period at 20°C after

high temperature treatment. These mean incubation periods ranged from 108.5 to 132.4 days, which shows that these eggs were still diapausing.

Alternating low and high temperatures cause eggs of N. fasciatus to undergo diapause and this effect increases with the decrease in duration at each temperature in each cycle. This characteristic could be of high survival value by enabling the eggs laid in the late summer to undergo dia-pause because of low night temperatures and high day temper-atures. This diapause is eventually broken by the oncoming long continuous cold winter. Eggs which were given four 12 day cycles of low and high temperatures have the same effect, that is, a very low incidence of diapausing eggs, as 24 days continuous low temperature treatment. Rakshpal (1962a) however, has shown that changing high and low temperatures (seven consecutive days each at 6 to 7°C, 23 to 26°C, 6 to 7°C, 23 to 26°C and fourteen days each at 5 to 6°C and 6 to 7°C) for 56 days, in eggs of Gryllus pennsylvanicus Burm. has the same effect on breaking diapause as 60 days continuous 6 to 7°C exposure. In other words 42 days low temperature treatment (the total number of days for which eggs remained at low temperature during the treatment) in changing high and low temperatures have the same effect as continuous 60 days chilling.

In N. fasciatus the total number of incubation days at high temperature in post-diapause eggs remains the same regardless of the time when these eggs were subjected

to low temperature again during their incubation and the
duration of that time of chilling.   Thus post-diapause
eggs in early summer continue to develop whenever tempera-
tures are favourable.   Low temperatures retard or prevent
development and eggs resume their normal development
shortly after the termination of low temperature periods.
Rakshpal (1962b) has shown somewhat similar results in
<u>Gryllus pennsylvanicus</u> Burm. but post-diapause eggs in this
case took three days to recover from the second chilling.

6.   EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON DIAPAUSE

6.1   Introduction

Since the classical work of Kogure (1933) on the influence of photoperiod on the life cycle of the silk worm, Bombyx mori L., diapause has been proved to be day-length dependent in a large number of insects and this number is ever increasing.   Most of the work done on insects has been on the influence of photoperiod on the commencement of facultative diapause in multivoltine species.   This aspect has been worked out in only a few species of univoltine insects, probably because it is believed that most univoltine species enter an obligatory diapause through which the seasonal rhythm of their life cycle is rigidly fixed.   It is possible that some of the univoltine species have their life cycle regulated by a photoperiodic clock because only the photoperiod is an accurate indicator of seasonal changes. Masaki (1963) studied the effect of photoperiod on the development of Nemobius yezoensis Shiraki, a univoltine Japanese cricket overwintering as late instar nymphs.   He found that nymphal diapause is terminated by long photoperiod (16 hours per day), but it is maintained for a long time by a short one (12 hours).   In the Orthoptera as a whole there are very few papers on the effects of photoperiod on diapause.   Some of the important ones are works of Norris (1959, 1965) on

red locust, <u>Nomadacris septemfasciata</u> (Serv.), Woodrow (1964)

on <u>Melanoplus devastator</u> Scudder, and Halliburton and

Alexander (1964) on <u>Chortophaga viridifasciata</u> (DeGeer).

  The present part of this study was undertaken with

the hope of determining whether the apparent mono-cyclic

life history of <u>N. fasciatus</u> at the latitude of Edmonton is

due to a short summer (in other words a short period of fav-

ourable conditions for development) or is regulated by the

effect of photoperiod on some stage in its life history,

causing the production of diapausing eggs. Experiments

were conducted to study the effect of photoperiod on the

breaking of diapause in eggs during low temperature treat-

ment; and on the various post-embryonic stages by obtaining

the incidence of diapause in the eggs laid by them. The

criteria for diapausing and non-diapausing eggs are the same

as in the previous sections.

6.2 <u>Influence of light on diapause during chilling</u>.

6.21 Introduction

  It is known that the duration of the diapause stage

can be affected by photoperiod in certain insects. Gayspitz

(1953, cited in Danilyevsky, 1965) found that diapause in the

larvae of the pine moth, <u>Dendrolimus pini</u> L. (Lepidoptera:

Lasiocampidae) under conditions of 12 hours daily light and

a temperature of 20°C lasts about a month, whereas at the same temperature with continuous illumination activity begins after 17 days. Danilyevsky (1965) also mentioned a similar but stronger effect of photoperiod on the dia- pausing larvae of <u>Arctia</u> <u>caia</u> L.   (Lepidoptera: Arctiidae) and <u>Parasemia</u> <u>plantaginis</u> L.   (Lepidoptera: Arctiidae).

<u>N.</u> <u>fasciatus</u> deposits eggs in the soil at a depth ranging from three to seven millimeters which could be an adaptation to counteract the possible diapause inhibiting effect of light. An experiment was devised to determine the influence of different daily photoperiods on the eggs during their exposure to low temperature.

6.22   Methods and materials

Five hundred eggs were taken from eggs laid within 24 hours in September 1964.   These eggs were divided into five groups of 100.   Another sample of 500 eggs was used in 1965 for replication of the experiment.   All these eggs were left at 22 $\pm$ 1°C in the dark for 10 days to allow them to reach the diapause stage.   Thereafter one group from each sample was kept under 8, 12, 16 and 24 hours daily light at 5 $\pm$ 1°C in light-proof compartments for 30 days.   The remaining fifth group was kept in total darkness to serve as a control.   The light in each treatment was provided by a 16 inch 15 w. fluorescent "cool white" lamp

operated by a time switch. The light intensity was about
85 - 90 foot candles on the shelf holding the petri dishes
containing the eggs. All the eggs were incubated at
29 ± 1°C after 30 days treatment at low temperature.

6.23   Results

Table 11 shows that the eggs in all the treatments
were virtually free from diapause. Although there is no
significant difference in percentage hatching among the
treatments, eight hours daily photoperiod showed slightly
better results (93 percent hatching) than the rest of the
photoperiods. This is probably just by chance. Thus it
appears that only low temperature breaks diapause in this
species.

6.3 The effects of photoperiod on diapause during chilling
     of eggs, and at different incubation temperatures.

6.31   Introduction

It was mentioned previously that the percentage
hatching of eggs of N. fasciatus is greater at higher in-
cubation temperatures than at lower ones, when they are in-
cubated without any low temperature treatment to break dia-
pause. Moreover, eggs remain at low temperatures for very

TABLE 11

The mean percentage hatching of eggs of <u>N. fasciatus</u> at 5°C
with different photoperiod treatments and then incubated at 29°C.

Light per day	Percentage hatching
0 hours	88.50
8 hours	93.00
12 hours	87.00
16 hours	88.00
24 hours	87.00

long periods (at least from the end of October to the end of April in the region of Edmonton). The effect of very long exposure of these eggs to low temperatures and their incubation at various temperatures to determine the degree to which diapause is terminated was therefore studied. Secondly, as pointed out in the results of the previous experiment, the effect of eight hours daily photoperiod during chilling needed further clarification. The present experimental study was designed to determine the effects of the above mentioned factors and their interactions.

6.32   Methods and materials

In this experiment two replicates each of 2,475 eggs were used. For this purpose, 300 crickets were collected on September 10, 1964, in the field and kept in 10 battery jars in the laboratory. Eggs for one replicate were collected from those deposited on September 11. These eggs were divided into 75 groups of 33. They were kept at $22 \pm 1°C$ for 10 days and thereafter moved to $5 \pm 1°C$. At this temperature 15 such groups were subjected to each of 0, 8, 12, 16 and 24 hours daily light. Three groups were removed each time from every sample of 15 groups after 6, 12, 24, 48 and 96 days. One group from each of these three was incubated at $29 \pm 1°C$ or $24 \pm 1°C$ or $20 \pm 2.5°C$ temperature. This experiment was repeated on the same dates in 1965 and

under the same conditions to replicate for analysis of variance. Unfortunately the temperature in the cabinet set for 20°C started fluctuating so widely when the experiment was already in progress during both years that the data for 20°C had to be excluded from the statistical analysis. An analysis of variance for a split plot design was used because eggs were subjected to different incubation temperatures in different cabinets.

Those eggs which hatched within 16 days of incubation at 24 ± 1°C were taken as non-diapausing since 14 to 16 days is the average incubation period at 24 ± 1°C for post-diapause eggs which have been subjected to low temperature after 10 days of an initial incubation at 22 ± 1°C temperature.

6.33   Results

Table 12 shows that the percent hatching for different photoperiod treatments at each of the two incubation temperatures for the same durations of chilling are not significantly different. Secondly, hatching increases with the increase in duration of low temperature treatment at both the incubation temperatures. At 29 ± 1°C however, there is rapid increase in percentage hatching up to 24 days of chilling when eggs are virtually free of diapause. For chilling durations longer than 24 days the percentage hatching at this temperature does not vary much.   On the

TABLE 12

The mean percentage hatching of eggs after exposure to different photoperiods at 5°C for different periods and then incubated at 24° or 29°C.

Exposure to 5°C in days	Hours light per day at 5°C									
	0		8		12		16		24	
	24°C	29°C	24°C	29°C	24°C	29°C	24°C	29°C	24°C	29°C
6	3.0	60.0	0.0	57.0	4.0	64.0	3.2	79.5	3.2	74.2
12	3.0	82.0	22.2	80.2	18.0	95.0	30.0	82.0	36.5	95.0
24	26.2	93.0	31.0	95.0	28.0	97.0	43.0	85.5	34.5	87.0
48	83.0	93.0	72.0	83.0	75.0	96.0	93.5	98.0	84.0	96.0
96	92.0	97.0	100.0	100.0	99.0	97.0	93.2	94.0	90.0	94.2

'F' value for different low temperature durations = 114.47**
         5% probability level = 2.87
         1% probability level = 4.43

'F' value for different incubation temperatures = 23.41**
         5% probability level = 4.23
         1% probability level = 7.72

other hand incubation at 24 ± 1°C shows very little breaking

of diapause by treatments up to 24 days at low temperature.

Diapause is terminated at an incubation temperature of

24 ± 1°C to a great extent when eggs had been chilled for

48 days. Ninety-six days chilling enabled almost all the

eggs to develop readily at this temperature.   This means

that the lower the incubation temperature the longer the

low temperature treatment required to allow the eggs to dev-

elop readily.

The observations from the previous experiment that

photoperiod during chilling does not affect termination of

diapause are confirmed from this experiment, because stat-

istically there is no significant difference among hatching

of eggs given different photoperiods.   The only significant

variations in hatching among the different treatments are

those which are due to different durations at low tempera-

ture, and due to different incubation temperatures. Both

these variations are highly significant.   It was found that

development in eggs proceeded normally at 20 ± 3°C when

chilled for 96 days.   It is possible, therefore, that eggs

after such a long chilling are capable of developing promptly

at lower incubation temperatures.

In conclusion it can be said that photoperiod does

not show any influence on eggs during their exposure to low

temperature and thus eggs are rendered free from diapause

only by low temperature. Secondly, eggs need long exposure
to low temperature for termination of their diapause to
develop at lower incubation temperatures.

6.4 The influence of light on post-embryonic stages

6.41   Introduction

There is much evidence showing the influence of
photoperiod on certain stages in the life history of dif-
ferent insects affecting diapause either in the succeeding
stages or in the next generation.   Dickson (1949) observed
in Grapholitha molesta (Busck.) in southern California that
a substantial proportion of the larvae entered diapause in
the early autumn when temperatures were still favourable
for development.   He found that diapause in these larvae
had already been determined by the photoperiod experienced
during the early larval instars.

Danilyevsky (1948, cited in Danilyevsky,1965)
found that if Acronycta rumicis L. (Lepidoptera: Noctuidae)
is exposed during the larval feeding period to a short day-
length (6 - 15 hours) virtually every individual enters dia-
pause in the pupal stage, whereas the incidence of diapause
fell to zero when the larvae experienced 17 hours daily
photoperiod.

Kogure (1933) showed in Bombyx mori L. that light

and temperature acting on eggs and early larval instars induced a particular pattern of diapause which appears in the eggs laid by the adults arising from these stages. As a result of development of the eggs and young larvae in a short-day regime (12 hours) at low temperatures (15°C), adults were obtained which laid pale non-diapausing eggs. On the other hand if development occurred in a long-day pattern (17 hours) about 70 percent of the moths laid diapausing eggs.

In all of the previous experiments on the influence of photoperiod on diapause in N. fasciatus only the egg and adult stages were considered. An experiment was, therefore, planned to determine whether photoperiod acting on the nymphs has any effect on the proportion of diapausing eggs deposited by the females arising from these nymphs.

6.42   Methods and material

Hatchlings for this experiment were obtained at 24 ± 1°C from post-diapause eggs which were deposited in September 1965. Nymphs were divided into 85 groups of ten, within 24 hours after hatching. Each group was reared up to maturity on rabbit pellets in a one pound wide-mouthed fruit jar covered with a fine insect screening lid. Each jar was provided with a continuous supply of water in an inverted vial and few paper towel strips. Five such jars were

exposed to 12 hours or 16 hours daily light in two growth chambers for each of the nymphal instars and adult stage. The growth chambers (Sherer) were equipped with regulated temperature and photoperiod. The light intensity on the surface holding the jars was approximately 500 foot candles and the temperature was kept at $24 \pm 1°C$. Since the mean durations of the six instars at $24 \pm 1°C$ are 5.8, 5.6, 4.7, 6.3, 8.1 and 9.8 days, respectively, the first instar was exposed for six days only, the second for six days only and so on to either 12 or 16 hours daily light. When not being exposed to light each instar group was placed in the dark. Five jars were subjected to each of 0, 12 and 16 hours daily light for all the post embryonic stages to serve as controls. All the jars were observed once a week to change the water vial. In the case of jars which were in darkness a 40 watt red light was used for observation purposes, but high mortality during handling resulted from this technique. After reaching the adult stage all the five samples of each treatment were transferred to a battery jar for convenience in getting eggs. For oviposition wet soil was provided in petri dishes in the usual manner on every alternative day and sometimes after every fourth day to minimize the exposure of adults to red light. Eggs thus obtained were sieved in water and incubated directly at $29 \pm 1°C$.

TABLE 13

The influence of photoperiod on different post-embryonic stages expressed as the percentage hatching of eggs laid by the resulting females. Incubation temperature was 29°C.

12 hours daily photoperiod				16 hours daily photoperiod			
Stage Illuminated	sex ratio		Percent Hatching at 29±1°C of their eggs	Stage Illuminated	Sex ratio		Percent hatching at 29±1°C of their eggs
	Males	Females			Males	Females	
Instar 1	6	5	21.7	Instar 1	8	7	51.3
Instar 2	5	7	30.0	Instar 2	13	16	48.0
Instar 3	11	8	43.0	Instar 3	9	6	44.2
Instar 4	10	8	48.5	Instar 4	7	15	43.3
Instar 5	9	8	46.0	Instar 5	12	5	28.3
Instar 6	8	9	45.2	Instar 6	8	13	27.7
Adult	5	15	41.7	Adult	5	16	23.5
All post-embryonic stages	2	5	37.0	All post-embryonic stages	6	8	21.0

Control crickets reared in total darkness had a sex ratio of 7 male and 5 female and 25% of their eggs hatched.

6.43   Results

Table 13 shows the sex ratio in each treatment and
the percentage hatching of the eggs at 29 ± 1°C laid by
the adults resulting from each treatment.   It is apparent
that either of the two photoperiods does not influence any
nymphal or adult stage to lay diapausing or non-diapausing
eggs because the percentage hatching of the eggs laid by
the control crickets reared in darkness throughout the post-
embryonic part of the life history is not much different
from the other treatments.   Only a small proportion of the
50 nymphs in each treatment reached maturity, which decreased
the precision of the results.

6.5 _Discussion_

The influence of photoperiod on the life cycle of
insects is not a very old aspect of ecology.   Marcovitch
(1923, cited in Danilyevsky, 1965) was probably the first
to discover the effect of photoperiod on the life cycle of
aphids.   It is only during the last few years that the sub-
ject of photoperiodism has attracted close attention.   In
most of the insects worked on so far, diapause has been found
to be influenced by photoperiod, but some insects have not
shown any response to photoperiod.   Danilyevsky and Gayspitz
(1948, cited in Lees, 1955) found _Phalera_ _bucephala_ (L.) and
Spilosoma _menthastri_ Esp. (Lepidoptera) behaving neutrally

to photoperiod concerning their diapause. Dickson (1949) also observed no effect of photoperiod on adult diapause in _Listroderes obliquus_ Klug. (Coleoptera) when larvae were grown under conditions of 9, 15 and 24 hours daily light, because in all the treatments almost every adult entered diapause. He also found _Lucilia sericata_ Meig. (Diptera) independent of photoperiod for its diapause.

N. fasciatus also is unaffected by photoperiod in any stage in regards to egg diapause under normal conditions. The independence of this cricket from photoperiod is further proved by the finding that no significant effect of 16 hours or 12 hours daily photoperiod on the various post-embryonic stages at $24 \pm 1°C$ rearing temperature was found in regard to incidence of diapause.

Up to the present in the Gryllidae the effects of temperature on breaking diapause has been mainly worked on. However, the photoperiod has been shown to be a significant factor in only one species, _Nemobius yezoensis_ Shiraki by Masaki (1963). He found this species to be a long-day form, that is, 16 hours daily light allows diapause-free development, whereas 12 hours induces diapause.

In _N. fasciatus_, the incubation temperature modified strongly the influence of the low temperature treatment, because diapause was found to be virtually broken at 29°C after 24 days chilling of eggs, whilst diapause was partially broken when the incubation temperature was $24 \pm 1°C$. Probably

the effect of low temperature on breaking diapause is enhanced by the following higher incubation temperature and this effect increases until an optimum temperature is reached.

Browning (1952b) also observed in Acheta commodus Walk. that the incubation temperature greatly influenced the termination of diapause after low temperature treatment. He found that 30 days exposure to 10.3°C results in 84 percent hatching at 29.9°C, whereas following the same low temperature treatment 64 percent hatched at 26.5°C and none at 20.9°C. He believed that a particular incubation temperature is required for diapause-free hatching of eggs. Diapause is terminated after a certain period of chilling, this period is longer if eggs are incubated at a lower temperature than at a higher incubation temperature.

These experiments were conducted to confirm the negative response of N. fasciatus to photoperiod which was shown by the effect of changing photoperiods under natural conditions and in the laboratory. Furthermore, the work was done to clarify whether this cricket reacts to constant photoperiods experienced by different post-embryonic stages, since large number of diapausing insects do react. The results obtained showed that photoperiod has no effect on induction or termination of diapause in this species.

These observations, however, should not be taken as valid for this species in general, because only the Edmonton strain is involved. For the generalization of these ob-

servations, more extensive experiments involving strains from

various geographical regions of its distribution will be

required.

7.   DISCUSSION

The term "diapause" was coined by Wheeler (1893) to describe an almost motionless stage of the embryo of the grasshopper _Xiphidium ensiferum_ Scudder after the completion of anatrepsis and before the commencement of katatrepsis. This term has since been used differently by many authors for the resting condition in all stages of insects. Steinberg and Kamensky (1936) introduced the terms "facultative" and "obligatory" to classify diapause into two clearly different-iated cases. Diapause is facultative when it is almost in-variably under the influence of environmental conditions and therefore it appears only in some generations. This type of diapause occurs commonly in bi- or multivoltine insects, as for example, _Gryllus veletis_ Alexander and Bigelow, _Acheta commodus_ Walk. and _Gryllus rubens_ Scudder (Bigelow, 1960). Diapause is obligatory when almost every individual of each generation enters diapause regardless of environmental conditions.   This is mostly the case with strictly univoltine insects such as _Gryllus pennsylvanicus_ Burm. (Bigelow, 1960 and Rakshpal, 1962a); and _Gryllus campestris_ Schrank (Bigelow, 1960).

The majority of egg-diapausing orthopterans such as _Dichromorpha viridis_ Scudder, _Chloealtis conspersa_ Harr., _Circotettix verruculatus_ Kirby, _Hesperotettix pratensis_ Scudder, _Hesperotettix viridis_, _Melanoplus differentialis_ Uhler,

M. femurrubrum DeGeer (Carothers, 1923), Aeropus sibiricus
(Bei-Benko, 1928), Chorthippus parallelus Zeit.(Sansome and
LaCour, 1935), Camnula pellucida Scudder (Moore, 1948),
Locustana pardalina (Walk.) (Matthee, 1951), Locusta migratoria
gallica Rem. (LeBerre, 1952), Acheta commodus Walk. (Hogan,
1960a), Gryllus pennsylvanicus Burm. (Rakshpal 1962a), under-
go diapause soon after the completion of anatrepsis. Most
of these orthopterans are almost strictly univoltine and
are inhabitants of geographical regions where favourable
conditions for development exist only for a short part of
the year. N. fasciatus like the above mentioned insects,under-
goes diapause at the close of anatrepsis. It is univoltine
in Alberta, which is probably also true for other localities
in southern Canada and the northern United States.

Individual variations in the degree of diapause occur
very commonly in most insects. Browning (1952b) found that
in Acheta commodus Walk. the proportion of the eggs entering
diapause depends upon the environment in which female was
living before oviposition. He did not, however, pinpoint
any particular factor which was responsible for these varia-
tions. In N. fasciatus wide variations were observed in the
proportion of diapausing eggs among eggs laid during suc-
ceeding summer weeks (Table 2). Wide fluctuations of temp-
eratures below 10 to above 20°C during the night and day
cause females to lay diapausing eggs, whereas narrow fluct-
uations with temperatures ranging from 15.5 to 27.7°C or
1.7 to 15.5°C influence laying of non-diapausing eggs. The

above fact was strongly supported by the diapause-inducing

effect of alternating high and low temperatures on the eggs

in the laboratory, since all the eggs entered diapause

within 48 days when they were exposed continuously to 24 hours

high temperature alternating with 24 hours low temperature.

It is therefore obvious that eggs during the pre- and post-

oviposition period are affected directly by external temp-

eratures. In order to undergo diapause eggs of N. fasciatus

require to be subjected to temperatures low enough (below

10°C) for breaking of diapause and high enough (above 20°C)

for normal development. The induction of diapause by

fluctuating temperatures enables all the eggs to enter dia-

pause by the end of summer. Thereby the possibility of un-

timely hatching of otherwise non-diapausing eggs in the same

summer, is greatly reduced   . This adaptation is, therefore,

of high survival value.

Rakshpal (1962a) also attempted to find the effect

of changing temperatures from low to high on the eggs in

Gryllus pennsylvanicus Burm. but he used seven day high temp-

erature exposure per 14 day cycle and 14 days per 28 day cycle,

in which he found that these changing temperatures are more

effective in breaking embryonic diapause than the continuous

low temperature treatment. Since his cycles of low and high

temperatures are not comparable to 24 hour or 48 hour cycles,

it is possible that daily changing temperatures may induce

rather than break diapause in Gryllus pennsylvanicus Burm.

There is very little information available at present con-
cerning the effects of daily fluctuations in temperatures on
diapause, since most of the work done so far has been on the
effects of continuous low and high temperatures on the break-
ing and induction of diapause.

A second probable factor contributing to variations
in the proportion of diapausing eggs, as mentioned previously,
is the age of the adult female at the time of oviposition.
It has been observed in Bombyx mori L. (Kogure, 1933),
Phlebotomus papatasii Scop. (Roubaud, 1935), Locustana pardalina
Walk. (Matthee, 1951), Lucilia sericata Meig. (Cragg and Cole,
1952), Gryllus pennsylvanicus Burm. (Rakshpal, 1962a) that
the physiological condition of the female at the time of
oviposition effects the presence or absence of diapause in
the next generation.

Lees (1955) states as a general rule that high temp-
eratures tend to avert diapause while low temperatures favour
arrest of development (p.29). This holds good in N. fasciatus
in which eggs without any treatment to break their diapause
gave higher percentage hatching at 29 $\pm$ 1°C as compared to
those at 22 $\pm$ 1°C or 20 $\pm$ 1.5°C. Browning (1952b) also
observed a similar case in the eggs of Acheta commodus Walk.
He found that as the incubation temperature was raised an
increasing percentage of the eggs developed without diapause
and that there was a strong tendency for diapause to be averted

at high temperatures. Bigelow (1960) also observed a similar case for nymphal diapause in _Gryllus veletis_ Alexander and Bigelow. There are several more examples from different insect orders (Lees, 1955), in which diapause is suppressed by the higher temperatures. In _N. fasciatus_ a high percentage hatching would not normally occur in the latitude of Edmonton because temperatures in late summer rarely rise above 25°C.

The termination of diapause in eggs of _N. fasciatus_ was found to be effected by low temperatures (0° - 15°C) followed by incubation temperature and this process was unaffected by changes in photoperiod at low temperatures. The effect of low temperature was noticed at 29°C incubation temperature even by chilling eggs for as short a period as six days. Eggs were found to require longer low temperature treatment for comparatively low incubation temperatures. Diapause was completely terminated for 20 - 29°C incubation temperatures after 96 days chilling at 5°C. Requirements of long low temperature treatment for diapause termination is in accordance of long winter in this latitude. Browning (1952b) also found in the eggs of _Acheta commodus_ Walk. that the proportion of the eggs that exhibited diapause was dependent upon the temperature at which the eggs were incubated after short duration of low temperature treatment.

The only morphological difference observed between diapausing and post-diapausing eggs of _N. fasciatus_ was that

the latter were clearer in their consistency. It is a general belief that during diapause some physiological changes occur which ultimately result in the resumption of active development. The difference in the consistency of diapausing and non-diapausing eggs in N. fasciatus is most likely due to some such physiological changes in the egg during low temperature treatment. Zolotarev (1947 and 1950, cited in Danilyevsky, 1965) calls these changes the diapause processes. Andrewartha (1952) introduced the term "diapause development" for the same thing. His term has since been used by other authors (Browning, 1952a,b; Lees, 1955; Masaki, 1962; Beck and Alexander, 1964a,b). Danilyevsky prefers the term "reactivation" for the same phenomenon, because he thinks that the term development is commonly linked with progressive growth and differentiation. Probably in these physiological changes different processes are involved in different insects (Harvey, 1962). Rakshpal (1962b) did not approve the use of the term diapause development in the egg diapause in Gryllus pennsylvanicus Burm. because he thinks that this term is confined to eggs exposed to the cold after reaching the diapause stage and not to pre-diapause eggs.

From all the experiments on the effect of photoperiod on the different stages of life history, it is obvious that photoperiod is not the decisive factor for inducing embryonic diapause in N. fasciatus. Temperature, however, is the chief factor inducing and terminating diapause, which is therefore,

the factor that decides the number of generations per year

in different geographical regions. Masaki (1961) mentioned

that in polyphagous species temperature determines the

length of the development period of an insect and hence

the annual number of generations. Voltinism varies from

place to place depending on the amount of effective temp-

erature.   Some species, therefore, change their annual

cycle from several generations to one generation with the

change in duration of unfavourable temperature from very

short to very long since their diapause behaviour is graded

from non-diapause to facultative or obligatory diapause from

lower latitudes to the higher ones. Bigelow (1960) observed

that diapause tendencies are more marked in the northern

than in the southern strain of _Gryllus fultoni_ Alexander.

It is probably also true for _N. fasciatus_ which is univoltine

in Alberta and multivoltine in Florida with intermediate

stages found at the localities in between.

It seems that _N. fasciatus_ does not have an adapt-

ive mechanism such as a very intense obligatory diapause or

diapause decided by the photoperiod to survive in this part

of the world. Diapause is facultative, because a certain

percentage of the eggs always hatch providing the incubation

temperature is above 26°C. Also, changes in temperature

influence the presence or absence of diapause in the eggs.

Under natural conditions, however, univoltinism is maintained,

firstly by the induction of diapause through alternations

of quite low temperatures at nights with fairly high temp-
eratures during days for most of the oviposition period
and secondly by temperatures in September and October being
too low for the completion of development before winter.
Therefore, even those eggs which need a short period of low
temperature for breaking diapause do not develop until
temperatures are high enough for a considerable length of
time.   The required high temperature would only be avail-
able in June and July of the following summer and by that
time diapause will be broken in all the eggs.   There is,
therefore, almost synchronous hatching in the first half
of July in nature.

8.　LITERATURE CITED

Alexander, R.D. and E.S. Thomas. 1959.　Systematic and be-
　　havioral studies on the crickets of the _Nemobius_ _fasciatus_
　　group (Orthoptera:　Gryllidae:　Nemobiinae).　Ann. ent.
　　Soc. Amer. 52(5):　591-605.

Allard, H.A. 1910.　Some New England Orthoptera observed in
　　late October.　Ent. News. 21(8):　352-7.

Andrewartha, H.G. 1943.　Diapause in the eggs of
　　Austroicetes _cruciata_ Sauss. (Acrididae), with partic-
　　ular reference to the influence of temperature on the
　　elimination of diapause.　Bull. ent. Res. 34:　1-17.

Andrewartha, H.G. 1952.　Diapause in relation to ecology of
　　insects.　Biol. Rev. 27:　50-107.

Andrewartha, H.G. and L.C. Birch. 1954.　The distribution
　　and abundance of animals.　The University of Chicago
　　Press,　Chicago, Illinois.

Ball, E.D., E.R. Tinkham, R. Flock and C.T. Vorhies. 1942.
　　Grasshoppers and other Orthoptera.　Tech. Bull. Univ-
　　ersity of Arizona.　93: pp.366.

Barber, G.W. and F.F. Dicke. 1939.　Effects of temperature
　　and moisture on overwintering pupae of the corn ear-
　　worm in the north-eastern states.　J. agric. Res. 59: 711-23.

Beck, S.D. and N. Alexander. 1964a. Chemically and photo-
periodically induced diapause development in the European
corn borer, Ostrinia nubilalis. Biol. Bull. mar. biol.
Lab., Woods Hole. 126: 174-184.

Beck, S.D. and N. Alexander. 1964b. Protodone, an insect
developmental hormone. Biol. Bull. mar. Biol. Lab.,
Woods Hole. 126: 185-198.

Beĭ-Benko, G.Y. 1928. Synopsis of the nymphs of the West
Siberian grasshoppers (in Russian with an English
summary). Trud. Sibirsk. Inst. S. Kh. Lesovodstova , pp. 9.

Bigelow, R.S. 1960. Developmental rates and diapause in
Acheta pennsylvanicus and Acheta veletis. Can. J. Zool.
38: 973-988.

Bigelow, R.S. 1962. Factors affecting developmental rates and
diapause in field crickets. Evolution 16: 396-406.

Blatchley, W.S. 1920. Orthoptera of North-eastern America.
The Nature Publishing Company, Indianapolis.

Browning, T.O. 1952a. The influence of temperature on com-
pletion of diapause in the eggs of Gryllulus commodus
Walk. (Orthoptera). Aus. J. sci. Res. Melbourne (B) 5:
112-127.

Browning, T.O. 1952b. On the rate of completion of diapause
development at constant temperatures in the eggs of
Gryllulus commodus Walk. (Orthoptera). Aus. J. sci.
Res. Melbourne (B) 5: 344-353.

Burdick, H.C. 1937. The effect of exposure to low tempera-
    ture on the developmental time of the embryos of the
    grasshopper, <u>Melanoplus</u> <u>differentialis</u> (Orthoptera).
    Physiol. Zool. 10: 156-70.

Cantrall, I.J. 1943. Orthoptera and Dermaptera of the
    George Reserve, Michigan. Mus. Zool. Univ. Michigan
    Misc. Publ. 54: pp. 182.

Carothers, E.E. 1923. Notes on taxonomy, development and life
    history of certain Acrididae. Trans. Amer. ent. Soc. 49:
    7-24.

Church, N.S. and R.W. Salt. 1952. Some effect of temperature
    on development and diapause in eggs of <u>Melanoplus</u> <u>bivittatus</u>.
    Can. J. Zool. 30: 173-84.

Corbet, P.S. 1956. Environmental factors influencing the
    induction and termination of diapause in the emperor
    dragonfly, <u>Anax</u> <u>imperator</u> (Odonata: Aeshnidae). J. exp.
    Biol. 33: 1-4.

Cousin, G. 1932. Etude expérimentale de la diapause des
    insects. Bull. biol. Suppl. 15: 1-341.

Cragg, J.B. and P. Cole. 1952. Diapause in <u>Lucilia</u> <u>sericata</u>.
    J. exp. Biol. 29: 600-604.

Danilevskii[*], A.S. 1965. Photoperiodism and seasonal devel-
    opment of insects. Oliver and Boyd, Edinburgh and
    London.

  [*] The same author as the one otherwise referred to as
    Danilyevsky.

DeGeer, Charles 1773. Mèmoires pour servir à L'Histoire des
    Insects III: pp. 522.

DeWilde, J. 1962. Photoperiodism in insects and mites. A.
    Rev. Ent. 7: 1-26.

Dickson, R.C. 1949. Factors governing the induction of dia-
    pause in the oriental fruit moth. Ann. ent. Soc. Amer.
    42: 511-37.

Fabricius, Johann C. 1775. Systema Entomologiae. p.281-2.

Fulton, B.B. 1931. A study of the genus Nemobius (Orthoptera:
    Gryllidae). Ann. ent. Soc.Amer. 24(2): 205-37.

Ghouri, A.S.K. and J.E. McFarlane. 1958. Observations on
    the development of crickets. Can. Ent. 90: 158-165.

Halliburton, W.H. and G. Alexander. 1964. Effect of photo-
    period on molting of Chortophaga viridifasciata (DeGeer)
    (Orthoptera: Acrididae). Ent. News. LXXV: 133-37.

Harris, T.H. 1841. Report on the insects of Massachusetts
    injurious to vegetation. VIII + 459 pp.

Harvey, W.R. 1962. Metabolic aspects of insect diapause. A.
    Rev. Ent. 7: 57-80.

Hebard, Morgan. 1930. Orthoptera of Alberta. Proceedings
    of Natural Science Philadelphia LXXXII: 377-403.

Hebard, Morgan. 1936. Orthoptera of North Dakota. Agric.
    expt. Sta. Bull. 284.

Hogan, T.W. 1959. A rapid method of examining diapause of eggs of embryos of _Acheta commodus_. Nature, Lond. 183: 269.

Hogan, T.W. 1960a. The onset and duration of diapause in eggs of _Acheta commodus_. Aust. J. biol. Sci. 13: 14-29.

Hogan, T.W. 1960b. The effects of sub-zero temperatures on the embryonic diapause of _Acheta commodus_ Walk. Aus. J. biol. Sci. 13: 527-40.

Kogure, M. 1933. The influence of light and temperature on certain characters of silkworm, _Bombyx mori_. J. Dept. Agri. Kyushu Imp. Univ. 4: 1-93.

Kozhanchikov, I.V. 1949. The amplitude of the daily temperature fluctuation as a factor in larval development of the Chinese Oak Silkworm (_Antheraea pernyi_ Guer.) C.R. Acad. Sci. URSS, Moscow (N.S.) 67: 381-384.

Le Berre, J.R. 1952. Le Phénomène de la blastocinèse dans les oeufs à diapause de _Locusta migratoria gallica_ (Rem.). C.R. Acad. Sci. Paris. 234: 1487-89.

Lees, A.D. 1955. The physiology of diapause in Arthropods. Cambridge Univ. Press, Cambridge.

Lugger, O. 1897. Grasshoppers, locusts, crickets, cockroaches, etc. of Minnesota. Agric. Expt. Sta. Bull. 55.

Masaki, S. 1961. Geographic variation of diapause in insects. Bull. Fac. Agric. Hirosaki Univ. 7: 66-98.

Masaki, S. 1962. The influence of temperature on the intensity of diapause in the eggs of the Emma field cricket (Orthoptera: Gryllidae). Kontyu. 30(1): 9-16.

Masaki, S. 1963. Photoperiodic control of growth and wing-form in Nemobius yezoensis Shiraki (Orthoptera: Gryllidae). Kontyu 31(1): 16-26.

Matthée, J.J. 1951. The structure and physiology of the egg of Locustana pardalina (Walk.). Sci. Bull. Dept. Agri. S. Afr. 316: 1-83.

Moore, H.W. 1948. Variations in fall embryological development in three grasshopper species. Can. Ent. 80: 83-88.

Norris, M.J. 1959. The influence of day length on imaginal diapause in the red locust, Nomadacris septemfasciata (Serv.). Ent. Exp. Appl. 2: 154-168.

Norris, M.J. 1965. The influence of constant and changing photoperiods on imaginal diapause in the red locust, Nomadacris septemfasciata (Serv.). J. Insect. Physiol. 11: 1105-19.

Parker, J.R. 1930. Some effects of temperature and moisture upon Melanoplus mexicanus mexicanus Sauss. and Camnula pellucida Scudder.(Orthoptera). Bull. Montana Agric. Exp. St. 223, 132 pp.

Pierce, G. 1948. The songs of insects; with related material on the production, propagation, detection and measurement of sonic and supersonic vibrations. Cambridge, Mass. Harvard University Press. VI + 399 pp.

Rakshpal, R. 1962a. Diapause in the eggs of <u>Gryllus</u> <u>pennsylvanicus</u>
     Burm. (Orthopera). Can. J. Zool. 40: 179-194.

Rakshpal, R. 1962b. Effect of cold on pre- and post-diapause
     eggs of <u>Gryllus</u> <u>pennsylvanicus</u> Burm. Proc. R. ent.
     Soc. Lond. (A) 37: 117-120.

Readio, P.A. 1931. Dormancy in <u>Reduvius</u> <u>personatus</u> L. Ann.
     ent. Soc. Amer. 24: 19-39.

Roubaud, E. 1935. Vie Latente et condition hibernale
   Provoquées par les influences maternalles chez certains
     invertébrés. Ann. sci. nat. 18: 39-51.

Sansome, F.W. and L. LaCour. 1935. The genetics of the grass-
     hopper, <u>Chorthippus</u> <u>parallelus</u>. J. Genet. 30: 415-22.

Scudder, S.H. 1868. Notes on the stridulation of some
     New England Orthoptera. Proc. Boston Soc. Nat. Hist.
     11: 2-7.

Scudder, S.H. 1877. New forms of saltatorial Orthoptera
     from the southern United States. Proc. Boston Soc.
     Nat. Hist. 19: 35-41.

Steinberg, D.M. and S.A. Kamensky. 1936. Les Premisses
     oecologiques de la diapause de <u>Loxostege</u> <u>sticlicalis</u> L.
     (Lepidoptera: Pyralidae) Bull. biol. Fr. Belg. 70:145

Strohecker, H.F. 1937. An ecological study of some Orthoptera
     of the Chicago area. Ecology 18 : 231-50.

Vickery, V.R. 1963. The occurrence of <u>Nemobius</u> <u>fasciatus</u>
     (DeGeer) in Newfoundland (Orthoptera: Gryllidae:
     Nemobiinae). Can. Ent. 95: 17.

Wheeler, W.M. 1893. A contribution to insect embryology.

    J. Morphol. 8: 1-160.

Woodrow, W. Middlekauff, 1964. Effects of photoperiod upon

    oogenesis in <u>Melanoplus</u> <u>devastator</u> Scudder. J. Kansas

    ent. Soc. 37:   163-168.

World Weather Records. 1941-50. 1959. U.S. Department of

    Commerce, Washington, D.C.